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The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) **Recombinant chimeric proteins and methods of use thereof**

(57) A chimeric protein having at least one domain derived from a physiologically active moiety and at least one domain derived from an extracellular matrix protein is provided. Physiologically active domains are derived from physiologically active moieties such as bone morphogenic proteins, transforming growth factors, and dermatan sulfate proteoglycans. The extracellular matrix protein domains are derived from collagen, fibrin, fibronectin, laminins and the like. Recombinant DNA constructs, cloning vectors and transformed cells containing DNA which encodes such chimeric proteins are provided. Methods of using the chimeric proteins, chimeric DNA constructs, cloning vectors containing chimeric DNA construct, and cells transformed with the cloning vectors are also provided. The chimeric proteins can be used as osteogenic agents and/or antiscarring agents.

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Description

BACKGROUND

1. Technical Field

Chimeric proteins and more particularly chimeric proteins having a domain which is derived from a physiologically active moiety and a domain derived from an extracellular matrix protein moiety are provided. Further provided are DNA constructs encoding such chimeric proteins and to methods for preparing such chimeric proteins using recombinant DNA technology. Methods for healing tissue including inducing scar reduction and formation of bone and/or cartilage are also provided.

2. Description of Related Art

Chimeric proteins, also known as fusion proteins, are hybrid proteins which combine two or more precursor proteins or peptides through peptide bonds. Fusion proteins may be produced by recombinant technology, i.e., by fusing part of the coding sequence of one gene to the coding sequence of another gene. The fused gene may then be used to transform a suitable organism which then expresses the fusion protein. Such proteins are usually used to test the function of different domains of a protein molecule or to append a locator or binding peptide onto a protein or peptide of interest. For example, portions upstream and partially downstream of human, rat or mouse collagen genes have been fused to other proteins in an attempt to analyze characteristics of transcription. See, e.g., Rossouw, et al. DNA Sequences in the First Intron of the Human ProAlpha-1(I) Collagen Gene Enhance Transcription, *Journal of Biological Chemistry*, 262 (31), pp. 15151-15157 (1987). Genomic imprinting effects have been analyzed by fusing the gene encoding human keratin 18 9 protein with the gene encoding beta-galactosidase (LacZ). See Thorex et al., Parent-Specific Expression of a Human Keratin 18/beta-galactosidase Fusion Gene in Transgenic Mice, *Dev. Dyn. (United States)*, 195 (2) pp. 100-12 (Oct. 1992). European Patent Application 88302039 describes production and purification of a recombinant protein, e.g., collagen, a linker region which may encode a restriction site, and a binding protein for a substrate. The fusion protein is then contacted with a suitable substrate to which it binds and the protein may then be recovered, e.g., from a column.

Extracellular matrix proteins ("EMPs") are found in spaces around or near cells of multicellular organisms and are typically fibrous proteins of two functional types: mainly structural, e.g., collagen and elastin, and mainly adhesive, e.g., fibronectin and laminin. Collagens are a family of fibrous proteins typically secreted by connective tissue cells. Twenty distinct collagen chains have been identified which assemble to form a total of about ten different collagen molecules. A general discussion of collagen is provided by Alberts, et al., *The Cell*, Garland Publishing, pp. 802-823 (1989), incorporated herein by reference. Other fibrous or filamentous proteins include Type I IF proteins, e.g., keratins; Type II IF proteins, e.g., vimentin, desmin and glial fibrillary acidic protein; Type III IF proteins, e.g., neurofilament proteins; and Type IV IF proteins, e.g., nuclear laminins.

Physiologically active glycoproteins, proteins, peptides and proteoglycans are abundant in living things. Such glycoproteins, proteins, peptides and proteoglycans are involved in a diverse array of cellular or viral functions which include initiation or regulation of metabolism, catabolism, reproduction, growth and repair of various life forms. Physiologically active glycoproteins, proteins, peptides, and proteoglycans include therapeutically active glycoproteins, proteins, peptides, and proteoglycans such as hormones, growth factors, enzymes, ligands and receptors and fragments thereof. Therapeutically active substances include glycoproteins, proteins, peptides and proteoglycans which have been used in medicine and research, e.g., to achieve a beneficial result in relation to disease states, trauma and/or to increase efficiency of normal cellular functions. Examples of therapeutically active glycoproteins, proteins, peptides and proteoglycans include cellular regulatory factors such as interleukins, GCSF, erythropoietin, insulin, growth hormone, ACTH, thyroid hormones, various growth factors, osteogenic or osteoinductive factors, decorin and the like.

Osteogenic agents are any of a family of proteins or peptides that induce formation of bone and/or cartilage. Osteogenin, bone morphogenic protein ("BMP") or osteoinductive protein are other terms which describe proteins having bone inducing activity. BMPs are a family of related proteins that trigger the developmental cascade of bone differentiation by inducing mesenchymal stem cells to grow into a variety of tissues including bone, cartilage, and dentin. The activity of BMPs is particularly useful for repairing large bone defects which may not heal without clinical intervention.

Osteogenic agents have been isolated from demineralized mammalian bone tissue (see, e.g., U.S. Patent Nos. 4,294,753 and 4,761,471). Substantially pure BMPs have been produced by recombinant DNA techniques (see, e.g., U.S. Patent Nos. 5,106,748, 5,187,076, 5,141,905, 5,108,922, 5,166,058, and 5,116,738). U.S. Patent No. 5,168,050 describes the use of a DNA construct having a DNA sequence encoding the precursor portion of BMP-2A ligated to a DNA sequence encoding BMP-2B for obtaining improved expression of BMP-2B.

Certain methods have been employed for inducing formation of bone and/or cartilage with BMPs. When BMP is implanted in viable tissue without a delivery formulation, the BMP resorbs rapidly and does not effectively induce bone formation. Therefore, formulations for delivery or implantation of BMPs have been developed.

The following are examples of attempts to make delivery devices for BMPs. U.S. Patent No. 4,472,840 describes collagen and BMP conjugates or complexes in the form of microporous sponges to induce the formation of osseous tissue in animals or humans. U.S. Patent No. 4,975,527 describes enzyme-solubilized collagen as a carrier of bone morphogenic protein. U.S. Patent No. 4,563,489 describes delivery systems for BMP that are admixtures of biodegradable organic polymers such as polylactic acid and polyglycolic acid.

U.S. Patent No. 5,106,626 describes administration of osteogenic protein extracted from mammalian bone admixed with or absorbed on a matrix such as tricalcium phosphate, hydroxyapatite, thermoplastic polymer materials, collagen, plaster of paris, polylactic acid, polycaprolactic acid, or polyglycolic acid. U.S. Patent Nos. 5,011,691 and 5,250,302 describe methods of purifying osteogenic protein from mammalian bone and combining it with a matrix of porous material such as collagen, homopolymers or copolymers of glycolic acid and lactic acid, hydroxyapatite, or tricalcium phosphate.

It has been suggested that to prevent rapid resorption of BMP from a site of implantation, osteogenic sequestering agents may be used in connection with an admixture of osteogenic protein and a porous polymeric matrix. U.S. Patent No. 5,171,579 describes a composition of an admixture of an osteogenic protein, a porous particulate matrix and an osteogenic protein sequestering amount of blood clot. PCT WO 93/00050 describes an admixture of an osteogenic protein, a polymer matrix of poly (lactic acid), poly (glycolic acid), and copolymers of lactic acid and glycolic acid, and an osteogenic protein-sequestering material which may be alkylcellulose, hyaluronic acid, alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer, poly(vinyl alcohol) or carboxymethylcellulose.

Notwithstanding the research done in the area of drug delivery devices, compositions which deliver a clinically effective dose of therapeutic agents over a predetermined period of time to precise target sites that combine easy handling for the medical practitioner with manufacturing convenience are still desirable. Elimination of the above-mentioned separate purified matrix materials, sequestering agents and substitution of more effective therapeutically active compositions would be advantageous.

SUMMARY

Chimeric proteins having a domain derived from at least one extracellular matrix protein and a domain derived from at least one cellular regulatory factor are provided. Suitable domains derived from cell regulatory factors include osteogenic domains, domains derived from a transforming growth factor, and domains derived from dermatan sulfate proteoglycans.

Recombinant DNA constructs having DNA sequences encoding the above mentioned chimeric proteins are provided. Cloning vectors incorporating the above DNA constructs and cells transformed with the vectors are also provided. Therapeutic compositions incorporating the above-mentioned chimeric protein(s) and pharmaceutically acceptable vehicles are provided. For example, a drug delivery composition is provided which has a chimeric protein having a domain derived from a fibrous protein and a domain derived from a physiologically active glycoprotein, protein, peptide and/or proteoglycan.

Methods for preparing a DNA construct including a DNA sequence encoding a cell regulatory factor (such as an osteogenic agent, a transforming growth factor, and/or a dermatan sulfate proteoglycan) operably linked to a DNA sequence encoding an extracellular matrix protein are provided. Also provided are methods of manufacturing osteogenic/extracellular matrix, transforming growth factor/extracellular matrix, and/or dermatan sulfate proteoglycan/extracellular matrix chimeric proteins by transforming a cell with a suitable cloning vector including a DNA construct encoding the osteogenic/extracellular matrix chimeric protein, the transforming growth factor/extracellular matrix chimeric protein, or the dermatan sulfate proteoglycan/extracellular matrix chimeric protein, respectively, culturing the cell in a suitable culture medium and isolating the chimeric protein from the culture medium.

In other embodiments, methods for inducing formation of bone, soft tissue repair, and reducing scar formation involve contacting with a suitable locus an osteogenic chimeric protein, a soft tissue chimeric protein, or an anti-scarring chimeric protein are provided, respectively. Suitable osteogenic chimeric proteins have a domain derived from one or more osteogenic agents and a domain derived from one or more extracellular matrix proteins. Suitable soft tissue chimeric proteins have a domain derived from at least one transforming growth factor and a domain derived from one or more extracellular matrix proteins. Suitable anti-scarring chimeric proteins have a domain derived from dermatan sulfate proteoglycan and a domain derived from one or more extracellular matrix proteins. Further provided are methods for inducing bone formation, soft tissue repair, and reducing scar formation by contacting the osteogenic chimeric protein, the soft tissue chimeric protein, or the anti-scarring chimeric protein, respectively, with an implant at a suitable locus in viable tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a nucleic acid sequence which encodes a BMP2B/collagen IA protein construct.

Fig. 2 depicts a nucleic acid sequence which encodes a transforming growth factor β /collagen IA protein construct.

Fig. 3 depicts a nucleic acid sequence which encodes a dermatan sulfate proteoglycan/collagen IA protein construct.

Fig. 4 depicts a nucleic acid sequence which encodes a dermatan sulfate proteoglycan peptide/collagen IA protein construct.

Fig. 5 depicts an amino acid sequence for a BMP2B/collagen IA chimeric protein.

Fig. 6 depicts an amino acid sequence for a TGF β /collagen IA chimeric protein.

Fig. 7 depicts an amino acid sequence for a dermatan sulfate proteoglycan/collagen IA chimeric protein.

Fig. 8 depicts an amino acid sequence for a dermatan sulfate proteoglycan peptide/collagen IA chimeric protein.

Fig. 9 depicts a pMal cloning vector containing a polylinker cloning site.

Fig. 10 depicts a polylinker cloning site contained in a pMal cloning vector of Fig. 9.

Fig. 11 depicts a pMal cloning vector containing a BMP2B/collagen IA DNA construct.

Fig. 12 depicts a pMal cloning vector containing a TGF β /collagen IA DNA construct.

Fig. 13 depicts a pMal cloning vector containing a decorin/collagen IA DNA construct.

Fig. 14 depicts a pMal cloning vector containing a decorin peptide/collagen IA DNA construct.

DETAILED DESCRIPTION

Chimeric proteins provide an integrated combination of a therapeutically active domain containing one or more therapeutically active moieties and an extracellular matrix protein domain containing one or more EMP moieties. The EMP domain provides an integral vehicle for delivery of the therapeutically active moiety to a target site. The two domains are linked covalently by one or more peptide bonds contained in a linker region. As used herein, integrated or integral means characteristics which result from the covalent association of one or more domains of the inventive chimeric proteins. The therapeutically active moieties disclosed herein are typically made of amino acids linked to form peptides, proteins, glycoproteins or proteoglycans.

The inherent characteristics of EMPs are ideal for use as a vehicle for the therapeutic moiety. Examples of suitable EMPs are collagen, elastin, fibronectin, and fibrin. Fibrillar collagens (Type I, II and III) assemble into ordered polymers and often aggregate into larger bundles. Type IV collagen assembles into sheetlike meshworks. Elastin molecules form filaments and sheets in which the elastin molecules are highly cross-linked to one another and provides good elasticity and high tensile strength. The cross-linked, random-coiled structure of the fiber network allows it to stretch and recoil like a rubber band. Fibronectin is a large fibril forming glycoprotein, which, in one of its forms, consists of highly insoluble fibrils cross-linked to each other by disulfide bonds. Fibrin is an insoluble protein formed from fibrinogen by the proteolytic activity of thrombin during the normal clotting of blood.

The molecular and macromolecular morphology of the above EMPs defines networks or matrices to provide substratum or scaffolding in integral covalent association with the therapeutically active moiety. The networks or matrices formed by the EMP domain provide an environment particularly well suited for ingrowth of autologous cells involved in growth, repair and replacement of existing tissue. The integral therapeutically active moieties covalently bound within the networks or matrices provide maximum exposure of the active agents to their targets to elicit a desired response.

Implants formed of or from the present chimeric proteins provide sustained release activity in or at a desired locus or target site. Unlike the above-described compositions discussed in the Background which incorporate a vehicle not covalently linked to an EMP, the therapeutically active domain of the present chimeric protein is not free to separately diffuse or otherwise be transported away from the vehicle which carries it, absent cleavage of peptide bonds. Consequently, chimeric proteins provide an effective anchor for therapeutic activity which allows the activity to be confined a target location for a prolonged duration. Because the supply of therapeutically active agent does not have to be replenished as often, smaller amounts of therapeutically active agent may be used over the course of therapy. Consequently, certain advantages provided by the inventive chimeric proteins are a decrease or elimination of local and systemic side effects, less potentiation or reduction in therapeutic activity with chronic use, and minimization of drug accumulation in body tissues with chronic dosing.

Use of recombinant technology allows manufacturing of nonimmunogenic chimeric proteins. The DNA encoding both the therapeutically active moiety and EMP moiety should preferably be derived from the same species as the patient being treated to avoid an immunogenic reaction. For example, if the patient is human, the therapeutically active moiety as well as the EMP moiety is preferably derived from human DNA.

Osteogenic/EMP chimeric proteins provide biodegradable and biocompatible agents for inducing bone formation at a desired site. In one embodiment a BMP moiety is covalently linked with an EMP to form a chimeric protein. The BMP moiety induces osteogenesis and the extracellular matrix protein moiety provides an integral substratum or scaffolding for the BMP moiety and cells which are involved in reconstruction and growth. Compositions containing the BMP/EMP chimeric protein provide effective sustained release delivery of the BMP moiety to desired target sites. The method of manufacturing such an osteogenic agent is efficient because the need for extra time consuming steps such as purifying EMP and then admixing it with the purified BMP are eliminated. An added advantage of the BMP/EMP chimeric protein results from the stability created by the covalent bond between BMP and the EMP, i.e., the BMP portion is not free to separately diffuse away from the EMP, thus providing a more stable therapeutic agent.

Bone morphogenic proteins are class identified as BMP-1 through BMP-9. A preferred osteogenic protein for use in human patients is human BMP-2B. A BMP-2B/collagen IA chimeric protein is illustrated in Fig. 5. The protein sequence illustrated in Fig. 5 includes a collagen helical domain depicted at amino acids 1-1057 and a mature form of BMP2B at amino acids 1060-1169. The physical properties of the chimeric protein are dominated in part by the EMP component. In the case of a collagen moiety, a concentrated solution of chimeric protein will have a gelatinous consistency that allows easy handling by the medical practitioner. The EMP moiety acts as a sequestering agent to prevent rapid desorption of the BMP moiety from the desired site and provide sustained release of BMP activity. As a results the BMP moiety remains at the desired site for a period of time necessary to effectively induce bone formation. The EMP moiety also provides a matrix which allows a patient's autologous cells, e.g., chondrocytes and the like, which are normally involved in osteogenesis to collect therein and form an autologous network for new tissue growth. The gelatinous consistency of the chimeric protein also provides a useful and convenient therapeutic manner for immobilizing active BMP on a suitable vehicle or implant for delivering the BMP moiety to a site where bone growth is desired.

The BMP moiety and the EMP moiety are optionally linked together by linker sequences of amino acids. Examples of linker sequences used are illustrated within the sequences depicted in Figs. 1-4 and described in more detail below. Linker sequences may be chosen based on particular properties which they impart to the chimeric protein. For example, amino acid sequences such as Ile-Glu-Gly-Arg and Leu-Val-Pro-Arg are cleaved by Factor Xa and Thrombin enzymes, respectively. Incorporating sequences which are cleaved by proteolytic enzymes into chimeric proteins provides cleavage at the linker site upon exposure to the appropriate enzyme and separation of the two domains into separate entities. It is contemplated that numerous linker sequences can be incorporated into any of the chimeric proteins.

In another embodiment, a chimeric DNA construct includes a gene encoding an osteogenic protein or a fragment thereof linked to a gene encoding an EMP or a fragment thereof. The gene sequences for various BMPs are known, see, e.g., U.S. Patent Nos. 4,294,753, 4,761,471, 5,106,748, 5,187,076, 5,141,905, 5,108,922, 5,166,058, 5,116,738 and 5,168,050, each incorporated herein by reference. A BMP-2B gene for use with this invention is synthesized by ligating oligonucleotides encoding a BMP protein. The oligonucleotides encoding BMP-2B are synthesized using an automated DNA synthesizer (Beckman Oligo-1000). In a preferred embodiment, the nucleotide sequence encoding the BMP is maximized for expression in *E. coli*. This is accomplished by using *E. coli* utilization tables to translate the sequence of amino acids of the BMP into codons that are utilized most often by *E. coli*. Alternatively, native DNA encoding BMP isolated from mammals including humans may be purified and used.

The BMP gene and the DNA sequence encoding an extracellular matrix protein are cloned by standard genetic engineering methods as described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor 1982, hereby incorporated by reference.

The DNA sequence corresponding to the helical region of collagen I(a) is cloned from a human fibroblast cell line. Two sets of polymerase chain reactions are carried out using cDNA prepared by standard methods from AG02261A cells. The first pair of PCR primers include a 5' primer bearing an XmnI linker sequence and a 3' primer bearing the BsmI site at nucleotide number 1722. The resulting PCR product consists of sequence from position 1 to 1722. The second pair of primers includes the BsmI site at 1722 and a linker sequence at the 3' end bearing a BglII site. The resulting PCR products consists of sequence from position 1722 to 3196. The complete helical sequence is assembled by standard cloning techniques. The two PCR products are ligated together at the BsmI site, and the combined clone is inserted into any vector with XmnI/BglII sites of XmnI-BamHI sites such as pMALc2-vector.

To clone the BMP-2B gene, total cellular RNA is isolated from human osteosarcoma cells (U-20S) by the method described by Robert E. Farrel Jr. (Academic Press, CA, 1993 pp.68-69) (herein incorporated by reference). The integrity of the RNA is verified by spectrophotometric analysis and electrophoresis through agarose gels. Typical yields of total RNA are 50 µg from a 100mm confluent tissue culture dish. The RNA is used to generate cDNA by reverse transcription using the Superscript pre-amplification system by Gibco BRL. The cDNA is used as template for PCR amplification using upstream and downstream primers specific for BMP-2B (GenBank HUMBMP2B accession # M22490). The resulting PCR product consists of BMP-2B sequence from position 1289-1619. The PCR product is resolved by electrophoresis through agarose gels, purified with gene clean (BIO 101) and ligated into pMal-c2 vector (New England Biolabs). The helical domain of human collagen I(a) chain is cloned in a similar manner. However, the total cellular RNA is isolated from a human fibroblast cell line (AG02261A human skin fibroblasts).

A chimeric BMP/EMP DNA construct is obtained by ligating a synthetic BMP gene to a DNA sequence encoding an EMP such as collagen, fibrin, fibronectin, elastin or laminin. However, the invention is not limited to these particular proteins. Fig. 1 illustrates a DNA construct which encodes a BMP-2B/collagen IA chimeric protein. The coding sequence for an EMP may be ligated upstream and/or downstream and in-frame with a coding sequence for the BMP. The DNA encoding an EMP may be a portion of the gene or an entire EMP gene. Furthermore, two different EMPs may be ligated upstream and downstream from the BMP.

The BMP-2B/collagen IA chimeric protein illustrated in Fig. 1 includes an XmnI linker sequence at base pairs (bp) 1-19, a collagen helical domain (bp 20-3190), a BglII/BamHI linker sequence (bp 3191-3196), a mature form of BMP-2B (bp 3197-3529) and a HindIII linker sequence (bp 3530-3535).

Any combination of growth factor and matrix protein sequences are contemplated including repeating units, or multiple arrays of each segment in any order. Incorporation of fragments of both matrix and growth factor proteins is also contemplated. For example, in the case of collagen, only the helical domain may be included. Other matrix proteins have defined domains, such as laminin, which has EGF-like domains. In these cases, specific functionalities can be chosen to achieve desired effects. Moreover, it may be useful to combine domains from disparate matrix proteins, such as the helical region of collagen and the cell attachment regions of fibronectin. In the case of growth factors, specific segments have been shown to be removed from the mature protein by post translational processing. Chimeric proteins can be designed to include only the mature biologically active region. For example, in the case of BMP-2B only the final 110 amino acids are found in the active protein.

In another embodiment, a transforming growth factor (TGF) moiety is covalently linked with an EMP to form a chimeric protein. The TGF moiety increases efficacy of the body's normal soft tissue repair response and also induces osteogenesis. Consequently, TGF/EMP chimeric proteins may be used for either or both functions. One of the fundamental properties of the TGFs is their ability to turn on various activities that result in the synthesis of new connective tissue. See, Piez and Sporn eds., *Transforming Growth Factor- β s Chemistry, Biology and Therapeutics*, Annals of the New York Academy of Sciences, Vol. 593, (1990). TGF- β is known to exist in at least five different isoforms. The DNA sequence for Human TGF- β_1 is known and has been cloned. See Derynck et al., *Human Transforming Growth Factor-Beta cDNA Sequence and Expression in Tumour Cell Lines*, *Nature*, Vol. 316, pp. 701-705 (1985), herein incorporated by reference. TGF- β_2 has been isolated from bovine bone, human glioblastoma cells and porcine platelets. TGF- β_3 has also been cloned. See ten Dijke, et al., *Identification of a New Member of the Transforming Growth Factor- β Gene Family*, *Proc. Natl. Acad. Sci. (USA)*, Vol. 85, pp. 4715-4719 (1988) herein incorporated by reference.

A TGF- β /EMP chimeric protein incorporates the known activities of TGF- β s and provides integral scaffolding or substratum of the EMP as described above to yield a composition which further provides sustained release focal delivery at target sites.

The TGF- β moiety and the EMP moiety are optionally linked together by linker sequences of amino acids. Linker sequences may be chosen based upon particular properties which they impart to the chimeric protein. For example, amino acid sequences such as Ile-Glu-Gly-Arg and Leu-Val-Pro-Arg are cleaved by Factor Xa and Thrombin enzymes, respectively. Incorporating sequences which are cleaved by proteolytic enzymes into the chimeric protein provides cleavage at the linker site upon exposure to the appropriate enzyme and separation of the domains into separate entities. Fig. 6 depicts an amino acid sequence for a TGF- β_1 /collagen IA chimeric protein. The illustrated amino acid sequence includes the collagen helical domain (1-1057) and a mature form of TGF- β_1 (1060-1171).

A chimeric DNA construct includes a gene encoding TGF- β_1 or a fragment thereof, or a gene encoding TGF- β_2 or a fragment thereof, or a gene encoding TGF- β_3 or a fragment thereof, ligated to a DNA sequence encoding an EMP protein such as collagen (I-IV), fibrin, fibronectin, elastin or laminin. A preferred chimeric DNA construct combines DNA encoding TGF- β_1 , a DNA linker sequence, and DNA encoding collagen IA. A chimeric DNA construct containing TGF- β_1 gene and a collagen IA gene is shown in Fig. 2. The illustrated construct includes an XmnI linker sequence (bp 1-19), DNA encoding a collagen helical domain (bp 20-3190), a BglII linker sequence (bp 3191-3196), DNA encoding a mature form of TGF- β_1 (3197-3535), and an XbaI linker sequence (bp 3536-3541).

The coding sequence for EMP may be ligated upstream and/or downstream and in-frame with a coding sequence for the TGF β . The DNA encoding the extracellular matrix protein may encode a portion of fragment of the EMP or may encode the entire EMP. Likewise, the DNA encoding the TGF- β may be one or more fragments thereof or the entire gene. Furthermore, two or more different TGF- β s or two or more different EMPs may be ligated upstream or downstream of alternate moieties.

In yet another embodiment, a dermatan sulfate proteoglycan moiety, also known as decorin or proteoglycan II, is covalently linked with an EMP to form a chimeric protein. Decorin is known to bind to type I collagen and thus affect fibril formation, and to inhibit the cell attachment promoting activity of collagen and fibrinogen by binding to such molecules near their cell binding sites. Chimeric proteins which contain a decorin moiety act to reduce scarring of healing tissue. The primary structure of the core protein of decorin has been deduced from cloned cDNA. See Krusius et al., *Primary Structure of an Extracellular Matrix Proteoglycan Core Protein-Deduced from Cloned cDNA*, *Proc. Natl. Acad. Sci. (USA)*, Vol. 83, pp. 7683-7687 (1986) incorporated herein by reference.

A decorin/EMP chimeric protein incorporates the known activities of decorin and provides integral scaffolding or substratum of the EMP as described above to yield a composition which allows sustained release focal delivery to target sites. Fig. 7 illustrates a decorin/collagen IA chimeric protein in which the collagen helical domain includes amino acids 1-1057 and the TGF- β mature protein includes amino acids 1060-1171. Fig. 8 illustrates a decorin peptide/collagen IA chimeric protein in which the collagen helical domain includes amino acids 1-1057 and the decorin peptide fragment includes amino acids 1060-1107. The decorin peptide fragment is composed of P46 to G93 of the mature form of decorin.

Further provided is a chimeric DNA construct which includes a gene encoding decorin or one or more fragments thereof, optionally ligated via a DNA linker sequence to a DNA sequence encoding an EMP such as collagen (I-IV), fibrin, fibronectin, elastin or laminin. A preferred chimeric DNA construct combines DNA encoding decorin, a DNA linker sequence, and DNA encoding collagen IA. A chimeric DNA construct containing a decorin gene and a collagen IA gene

is shown in Fig. 3. The illustrated construct includes an XmnI linker sequence (bp 1-19), DNA encoding a collagen helical domain (bp 20-3190), a BglII linker sequence (bp 3191-3196), DNA encoding a mature form of decorin (bp 3197-4186) and a PstI linker sequence. A chimeric DNA construct containing a decorin peptide gene and a collagen IA gene is shown in Fig. 4. The illustrated construct includes an XmnI linker sequence (bp 1-19), DNA encoding a collagen helical domain (bp 20-3190), a BglII linker sequence (bp 3191-3196), DNA encoding a peptide fragment of decorin (bp 3197-3343), and a PstI linker sequence (bp 3344-3349).

The coding sequence for an EMP may be ligated upstream and/or downstream and in-frame with a coding sequence for decorin. The DNA encoding the EMP may encode a portion or fragment of the EMP or may encode the entire EMP. Likewise, the DNA encoding decorin may be a fragment thereof or the entire gene. Furthermore, two or more different EMPs may be ligated upstream from the DNA encoding decorin moiety.

Any of the above described chimeric DNA constructs may be incorporated into a suitable cloning vector. Fig. 9 depicts a pMal cloning vector containing a polylinker cloning site. Preferred cloning vectors are the plasmids pMal-p2 and pMal-c2 (commercially available from New England Biolabs). The desired chimeric DNA construct is incorporated into a polylinker sequence of the plasmid which contains certain useful restriction endonuclease sites which are depicted in Fig. 10. The pMal-p2 polylinker sequence has XmnI, EcoRI, BamHI, HindIII, XbaI, SalI and PstI restriction endonuclease sites which are depicted in Fig. 10. The polylinker sequence is digested with an appropriate restriction endonuclease and the chimeric construct is incorporated into the cloning vector by ligating it to the DNA sequences of the plasmid. The chimeric DNA construct may be joined to the plasmid by digesting the ends of the DNA construct and the plasmid with the same restriction endonuclease to generate "sticky ends" having 5' phosphate and 3' hydroxyl groups which allow the DNA construct to anneal to the cloning vector. Gaps between the inserted DNA construct and the plasmid are then sealed with DNA ligase. Other techniques for incorporating the DNA construct into plasmid DNA include blunt end ligation, poly(dA.dT) tailing techniques, and the use of chemically synthesized linkers. An alternative method for introducing the chimeric DNA construct into a cloning vector is to incorporate the DNA encoding the extracellular matrix protein into a cloning vector already containing a gene encoding a therapeutically active moiety.

The cloning sites in the above-identified polylinker site allow the cDNA for the collagen IA/BMP-2B chimeric protein illustrated in Fig. 1 to be inserted between the XmnI and the HindIII sites. The cDNA encoding the collagen I/TGF- β_1 protein illustrated in Fig. 2 is inserted between the XmnI and the XbaI sites. The cDNA encoding the collagen IA/decorin protein illustrated in Fig. 3 is inserted between the XmnI and the PstI sites. The cDNA encoding the collagen IA/decorin peptide (dec 1) illustrated in Fig. 4 is inserted between the XmnI and PstI sites.

Plasmids containing the chimeric DNA construct are identified by standard techniques such as gel electrophoresis. Procedures and materials for preparation of recombinant vectors, transformation of host cells with the vectors, and host cell expression of polypeptides are described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor 1982 hereby incorporated by reference. Generally, prokaryotic or eukaryotic host cells may be transformed with the recombinant DNA plasmids. Transformed host cells may be located through phenotypic selection genes of the cloning vector which provide resistance to a particular antibiotic when the host cells are grown in a culture medium containing that antibiotic.

Transformed host cells are isolated and cultured to promote expression of the chimeric protein. The chimeric protein may then be isolated from the culture medium and purified by various methods such as dialysis, density gradient centrifugation, liquid column chromatography, isoelectric precipitation, solvent fractionation, and electrophoresis. However, purification of the chimeric protein by affinity chromatography is preferred whereby the chimeric protein is purified by ligating it to a binding protein and contacting it with a ligand or substrate to which the binding protein has a specific affinity.

In order to obtain more effective expression of mammalian or human eukaryotic genes in bacteria (prokaryotes), the mammalian or human gene should be placed under the control of a bacterial promoter. A protein fusion and purification system is employed to obtain the chimeric protein. Preferably, any of the above-described chimeric DNA constructs is cloned into a pMal vector at a site in the vector's polylinker sequence. As a result, the chimeric DNA construct is operably fused with the malE gene of the pMal vector. The malE gene encodes maltose binding protein (MBP). Fig. 11 depicts a pMal cloning vector containing a BMP/collagen DNA construct. A spacer sequence coding for 10 asparagine residues is located between the malE sequence and the polylinker sequence. This spacer sequence insulates MBP from the protein of interest. Figs. 12, 13 and 14 depict pMal cloning vectors containing DNA encoding TGF- β_1 , decorin and a decorin peptide, respectively. The pMal vector containing any of the chimeric DNA constructs fused to the malE gene is transformed into *E. coli*. This technique utilizes the PtaC promoter of the malE gene.

The *E. coli* is cultured in a medium which induces the bacteria to produce the maltose binding protein fused to the chimeric protein. The MBP contains a 26 amino acid N-terminal signal sequence which directs the MBP-chimeric protein through the *E. coli* cytoplasmic membrane. The protein can then be purified from the periplasm. Alternatively, the pMal-c2 cloning vector can be used with this protein fusion and purification system. The pMal-c2 vector contains an exact deletion of the malE signal sequence which results in cytoplasmic expression of the fusion protein. A crude cell extract containing the fusion protein is prepared and poured over a column of amylose resin. Since MBP has an affinity for the amylose it binds to the resin. Alternatively, the column can include any substrate for which MBP has a specific affinity. Unwanted proteins present in the crude extract are washed through the column. The MBP fused to the chimeric protein

is eluted from the column with a neutral buffer containing maltose or other dilute solution of a desorbing agent for displacing the hybrid polypeptide. The purified MBP-chimeric protein is cleaved with a protease such as factor Xa protease to cleave the MBP from the chimeric protein. The pMal-p2 plasmid has a sequence encoding the recognition site for protease factor Xa which cleaves after the amino acid sequence Isoleucine-Glutamic acid-Glycine-Arginine of the polylinker sequence.

The chimeric protein is then separated from the cleaved MBP by passing the mixture over an amylose column. An alternative method for separating the MBP from the chimeric protein is by ion exchange chromatography. This system yields up to 100mg of MBP-chimeric protein per liter of culture. See Riggs, P., in Ausebel, F.M., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (eds.) Current Protocols in Molecular Biology, Supplement 19 (16.6.116.6.10) (1990) Green Associates/Wiley Interscience, New York, New England Biolabs (cat # 800-65S 9pMALc2) pMal protein fusion and purification system hereby incorporated by reference. (See also European Patent No. 286 239 herein incorporated by reference which discloses a similar method for production and purification of a protein such as collagen.)

Other protein fusion and purification systems may be employed to produce chimeric proteins. Prokaryotes such as *E. coli* are the preferred host cells for expression of the chimeric protein. However, systems which utilize eukaryote host cell lines are also acceptable such as yeast, human, mouse, rat, hamster, monkey, amphibian, insect, algae, and plant cell lines. For example, HeLa (human epithelial), 3T3 (mouse fibroblast), CHO (Chinese hamster ovary), and SP 2 (mouse plasma cell) are acceptable cell lines. The particular host cells that are chosen should be compatible with the particular cloning vector that is chosen.

Another acceptable protein expression system is the Baculovirus Expression System manufactured by Invitrogen of San Diego, California. Baculoviruses form prominent crystal occlusions within the nuclei of cells they infect. Each crystal occlusion consists of numerous virus particles enveloped in a protein called polyhedrin. In the baculovirus expression system, the native gene encoding polyhedrin is substituted with a DNA construct encoding a protein or peptide having a desired activity. The virus then produces large amounts of protein encoded by the foreign DNA construct. The preferred cloning vector for use with this system is pBlueBac III (obtained from Invitrogen of San Diego, California). The baculovirus system utilizes the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) regulated polyhedrin promoter to drive expression of foreign genes. AcMNPV is isolated from a moth called the California looper. The chimeric gene, i.e., the DNA construct encoding the chimeric protein, is inserted into the pBlueBac III vector immediately downstream from the baculovirus polyhedrin promoter.

The pBlueBac III transfer vector contains a B-galactosidase reporter gene which allows for identification of recombinant virus. The B-galactosidase gene is driven by the baculovirus ETL promoter (PETL) which is positioned in opposite orientation to the polyhedrin promoter (PpH) and the multiple cloning site of the vector. Therefore, recombinant virus coexpresses B-galactosidase and the chimeric gene.

Spodoptera frugiperda (Sf9) insect cells are then cotransfected with wild type viral DNA and the pBlueBac III vector containing the chimeric gene. Recombination sequences in the pBlueBac III vector direct the vector's integration into the genome of the wild type baculovirus. Homologous recombination occurs resulting in replacement of the native polyhedrin gene of the baculovirus with the DNA construct encoding the chimeric protein. Wild type baculovirus which do not contain foreign DNA express the polyhedrin protein in the nuclei of the infected insect cells. However, the recombinants do not produce polyhedrin protein and do not produce viral occlusions. Instead, the recombinants produce the chimeric protein.

Alternative insect host cells for use with this expression system are Sf21 cell line derived from *Spodoptera frugiperda* and High Five cell lines derived from *Trichoplusia ni*.

Other acceptable cloning vectors include phages, cosmids or artificial chromosomes. For example, bacteriophage lambda is a useful cloning vector. This phage can accept pieces of foreign DNA up to about 20,000 base pairs in length. The lambda phage genome is a linear double stranded DNA molecule with single stranded complementary (cohesive) ends which can hybridize with each other when inside an infected host cell. The lambda DNA is cut with a restriction endonuclease and the foreign DNA, e.g. the DNA to be cloned, is ligated to the phage DNA fragments. The resulting recombinant molecule is then packaged into infective phage particles. Host cells are infected with the phage particles containing the recombinant DNA. The phage DNA replicates in the host cell to produce many copies of the desired DNA sequence.

Cosmids are hybrid plasmid/bacteriophage vectors which can be used to clone DNA fragments of about 40,000 base pairs. Cosmids are plasmids which have one or more DNA sequences called "cos" sites derived from bacteriophage lambda for packaging lambda DNA into infective phage particles. Two cosmids are ligated to the DNA to be cloned. The resulting molecule is packaged into infective lambda phage particles and transfected into bacteria host cells. When the cosmids are inside the host cell they behave like plasmids and multiply under the control of a plasmid origin of replication. The origin of replication is a sequence of DNA which allows a plasmid to multiply within a host cell.

Yeast artificial chromosome vectors are similar to plasmids but allow for the incorporation of much larger DNA sequences of about 400,000 base pairs. The yeast artificial chromosomes contain sequences for replication in yeast. The yeast artificial chromosome containing the DNA to be cloned is transformed into yeast cells where it replicates thereby producing many copies of the desired DNA sequence. Where phage, cosmids, or yeast artificial chromosomes

are employed as cloning vectors, expression of the chimeric protein may be obtained by culturing host cells that have been transfected or transformed with the cloning vector in a suitable culture medium.

Chimeric proteins disclosed herein are intended for use in treating mammals or other animals. The therapeutically active moieties described above, namely, osteogenic agents such as BMPs, TGFs, decorin, and/or fragments of each of them, are all to be considered as being or having been derived from cellular regulatory factors for purposes. The chimeric proteins and DNA constructs which incorporate a domain derived from one or more cellular regulatory factors can be used for *in vivo* therapeutic treatment, *in vitro* research or for diagnostic purposes in general.

When used in *vivo*, formulations containing the inventive chimeric proteins may be placed in direct contact with viable tissue, including bone, to induce or enhance growth, repair and/or replacement of such tissue. This may be accomplished by applying a chimeric protein directly to a target site during surgery. It is contemplated that minimally invasive techniques such as endoscopy are to be used to apply a chimeric protein to a desired location. Formulations containing the chimeric proteins disclosed herein may consist solely of one or more chimeric proteins or may also incorporate one or more pharmaceutically acceptable adjuvants.

In an alternate embodiment, any of the above-described chimeric proteins may be contacted with, adhered to, or otherwise incorporated into an implant such as a drug delivery device or a prosthetic device. Chimeric proteins may be microencapsulated or macroencapsulated by liposomes or other membrane forming materials such as alginic acid derivatives prior to implantation and then implanted in the form of a pouchlike implant. The chimeric protein may be microencapsulated in structures in the form of spheres, aggregates of core material embedded in a continuum of wall material or capillary designs. Microencapsulation techniques are well known in the art and are described in the Encyclopedia of Polymer Science and Engineering, Vol. 9, pp. 724 et seq. (1980) hereby incorporated by reference.

Chimeric proteins may also be coated on or incorporated into medically useful materials such as meshes, pads, felts, dressings or prosthetic devices such as rods pins, bone plates, artificial joints, artificial limbs or bone augmentation implants. The implants may, in part, be made of biocompatible materials such as glass, metal, ceramic, calcium phosphate or calcium carbonate based materials. Implants having biocompatible biomaterials are well known in the art and are all suitable for use. Implant biomaterials derived from natural sources such as protein fibers, polysaccharides, and treated naturally derived tissues are described in the Encyclopedia of Polymer Science and Engineering, Vol. 2, pp. 267 et seq. (1989) hereby incorporated by reference. Synthetic biocompatible polymers are well known in the art and are also suitable implant materials. Examples of suitable synthetic polymers include urethanes, olefins, terephthalates, acrylates, polyesters and the like. Other acceptable implant materials are biodegradable hydrogels or aggregations of closely packed particles such as polymethylmethacrylate beads with a polymerized hydroxyethyl methacrylate coating. See the Encyclopedia of Polymer Science and Engineering, Vol. 2, pp. 267 et seq. (1989) hereby incorporated by reference.

The chimeric protein provides a useful way for immobilizing or coating a cellular regulatory factor on a pharmaceutically acceptable vehicle to deliver the cellular regulatory factor to desired sites in viable tissue. Suitable vehicles include those made of bioabsorbable polymers, biocompatible nonabsorbable polymers, lactoner putty and plaster of Paris. Examples of suitable bioabsorbable and biocompatible polymers include homopolymers, copolymers and blends of hydroxyacids such as lactide and glycolide, other absorbable polymers which may be used alone or in combination with hydroxyacids include dioxanones, carbonates such as trimethylene carbonate, lactones such as caprolactone, polyoxalylkenes, and oxylates. See the Encyclopedia of Polymer Science and Engineering, Vol. 2, pp. 230 et seq. (1989) hereby incorporated by reference.

These vehicles may be in the form of beads, particles, putty, coatings or film vehicles. Diffusional systems in which a core of chimeric protein is surrounded by a porous membrane layer are other acceptable vehicles.

The following examples should be considered as illustrative of certain embodiments disclosed herein but should not be considered as limiting the inventive disclosure.

EXAMPLE I

Cloning BMP-2B/collagen IA DNA segment constructs

Obtaining PCR products for BMP-2B and Collagen I(a): The chimeric gene encoding the BMP-2B/Collagen I(a) fusion protein is assembled from PCR products. The PCR primers are designed to provide restriction sites on the 5' and 3' ends that facilitate later ligation steps. The 5' and 3' ends of the BMP-2B PCR product contain BamHI and HindIII restriction sites respectively. The 5' and 3' ends of the Collagen I(a) PCR product contain XmnI and BglII restriction sites respectively. Amplification is carried out on template cDNA synthesized from total cellular RNA using standard methods. PCR reactions for BMP-2B and Collagen I(a) use cDNA prepared from U-20S and AG02261A cell lines respectively. After amplification and purification, the PCR products are ligated into PCR II vectors. Positive clones are identified by screening plasmids for the correct molecular weight. The clones are verified by DNA sequencing using standard methods. The BMP-2B PCR product is excised from PCR II by restriction digestion with BamHI and HindIII and the Collagen I(a) segment was excised from PCR II using XmnI and BglII. The restriction digest reactions are resolved by electrophoresis

through agarose gels and the DNA fragments with the BMP-2B and Collagen I(a) sequences are purified with gene clean (BIO 101).

EXAMPLE II

Cloning TGF- β 1/collagen IA DNA segment constructs

Obtaining PCR products for TGF- β 1 and Collagen I(a): The chimeric gene encoding the TGF- β 1/Collagen I(a) fusion protein is assembled from PCR products. The PCR primers are designed to provide restriction sites on the 5' and 3' ends that facilitate later ligation steps. The 5' and 3' ends of the TGF- β 1 PCR product contain BglII and XbaI restriction sites respectively. The 5' and 3' ends of the Collagen I(a) PCR product contain XmnI and BglII restriction sites respectively. Amplification is carried out on template cDNA synthesized from total cellular RNA using standard methods. PCR reactions for TGF- β 1 and Collagen I(a) use cDNA prepared from AG02261A cells. After amplification and purification, the PCR products are ligated into PCR II vectors. Positive clones are identified by screening plasmids for the correct molecular weight. The clones are verified by DNA sequencing using standard methods. The TGF- β 1 PCR product is excised from PCR II by restriction digestion with BglII and XbaI and the Collagen I(a) segment was excised from PCR II using XmnI and BglII. The restriction digest reactions are resolved by electrophoresis through agarose gels and the DNA fragments with the TGF- β 1 and Collagen I(a) sequences are purified with gene clean (BIO 101).

EXAMPLE III

Cloning dermatan sulfate proteoglycan (decorin)/collagen IA DNA segment constructs

Obtaining PCR products for Decorin and Collagen I(a): The chimeric gene encoding the Decorin/Collagen I(a) fusion protein is assembled from PCR products. The PCR primers are designed to provide restriction sites on the 5' and 3' ends that facilitate later ligation steps. The 5' and 3' ends of the Decorin PCR product contain BamHI and PstI restriction sites respectively. The 5' and 3' ends of the Collagen I(a) PCR product contain XmnI and BglII restriction sites respectively. Amplification is carried out on template cDNA synthesized from total cellular RNA using standard methods. PCR reactions for Decorin and Collagen I(a) use cDNA prepared from AG02261A cells. After amplification and purification, the respective PCR products are ligated into respective PCR II vectors. Positive clones are identified by screening plasmids for the correct molecular weight. The clones are verified by DNA sequencing using standard methods. The Decorin PCR product is excised from PCR II by restriction digestion with BamHI and PstI and the Collagen I(a) segment was excised from PCR II using XmnI and BglII. The restriction digest reactions are resolved by electrophoresis through agarose gels and the DNA fragments with the Decorin and Collagen I(a) sequences are purified with gene clean (BIO101).

EXAMPLE IV

Construction of cloning vector incorporating DNA constructs of Example 1

Ligation of BMP-2B and Collagen I(a) segments into the pMal-c2 expression vector: The pMal-c2 vector is treated with BamHI and Hind3, resolved by electrophoresis through an agarose gel and purified by standard methods. The BMP-2B segment with matching BamHI and Hind3 restriction sites on the 5' and 3' ends is ligated into pMal-c2 and transformants are screened for the insert by standard techniques. Positive clones are verified by DNA sequencing and designated pMal-c2 BMP. To complete the construction, pMal-c2-BMP is digested with XmnI and BamHI and the Collagen I(a) segment which is digested with XmnI and BglII is ligated into those sites by standard methods (BamHI and BglII produce compatible termini). Positive clones are verified by DNA sequencing and designated pMal-CB. See Fig. 11.

EXAMPLE V

Construction of cloning vector incorporating DNA constructs of Example II

Ligation of TGF-B1 and Collagen I(a) segments into the pMal-c2 expression vector: The pMal-c2 vector is treated with XmnI and XbaI, resolved by electrophoresis through an agarose gel and purified by standard methods. The Collagen I(a) segment with a 5' XmnI site and a 3' BglII restriction site and the TGF-B1 segment with a 5' BglII site and a 3' XbaI site are combined with the digested and purified pMal-c2 plasmid for a three fragment ligation reaction using standard methods. Transformants are screened for the insert by standard techniques. Positive clones are verified by DNA sequencing and designated pMal-CT. See Fig. 12.

EXAMPLE VI

Construction of cloning vector incorporating DNA constructs of Example III

5 Ligation of Decorin and Collagen I(a) segments into the pMal-c2 expression vector: The pMal-c2 vector is treated with XmnI and PstI, resolved by electrophoresis through an agarose gel and purified by standard methods. The Collagen I(a) segment with a 5' XmnI site and a 3' BglII restriction site and the Decorin segment with a 5' BamHI site and a 3' PstI site are combined with the digested and purified pMal-c2 plasmid for a three fragment ligation reaction using standard methods (BamHI and BglII produce compatible termini). Transformants are screened for the insert by standard techniques. Positive clones are verified by DNA sequencing and designated pMal-CD. See Fig. 13.

EXAMPLE VII

Transformation of E. Coli and Expression of a Collagen/TGF- β 1 and Collagen/Decorin Chimeric Genes in E. coli

15 Expression plasmids pMal-CB (Collagen-BMP2B Chimera), pMal-CT (Collagen-TGF- β 1 Chimera) and pMal-CD (Collagen-Decorin Chimera) are used to transform E.coli HB 101 using standard techniques. To express protein, a 50 ml culture of E.coli harboring one of the expression vectors is inoculated into 1L of LB broth and incubated with agitation at 37°C. When the A_{600} is 0.5 ± 0.1 , 0.1M IPTG is added to a final concentration of 1.5-15 mM. The culture is maintained at 37°C until the A_{600} is 1.3 to 1.8 and the E.coli is harvested by centrifugation at 4000xg. The cell pellets are resuspended in 7.5 ml 20 mM Tris HCl pH 7.5, 200 mM NaCl, 1 mM EDTA (hereinafter "column buffer") and frozen in a dry ice/ethanol bath. The frozen cell pellets are thawed at 4°C, then sonicated on ice until the cells are disrupted. Cell debris is removed by centrifugation at 9,000xg at 4°C for 30 minutes. The supernatant fraction contains the E.coli crude cell lysate which is analyzed for protein production by SDS-PAGE. The recombinant protein products produced from these pMal vectors is a fusion protein with MBP (maltose binding protein). The MBP segment is included to allow a single step purification of the protein.

20 The crude lysate is passed over an amylose column containing ml of resin/3 mg of recombinant protein (expected yield). The column is washed with 8 volumes of column buffer and the column flow through is reapplied to the column. Another 8 volumes of column buffer is used to wash the column. The fusion protein is eluted from the column using column buffer containing 10 mM Maltose. Fractions containing the recombinant chimeric protein are identified by the BCA protein assay (Pierce) and verified by SDS-PAGE. The fractions that contain the protein are pooled

30 The MBP segment of the purified protein is cleaved from the collagen-growth factor chimera by treatment with factor Xa (New England Biolabs) at room temperature for 24 hours. The collagen-growth factor chimera is separated from the MBP segment by chromatography through an amylose column. The column flow through contains the collagen-growth factor chimera, which is analyzed by SDS-PAGE. Typical yield of purified protein range from 10-50 mg/liter of E.coli culture.

EXAMPLE VIII

40 Expression of a Collagen-Growth Factor Chimeric Genes in Sf9 Cells

A useful alternative to the E.coli expression system is Baculovirus. The gene for the collagen-growth factor chimeras is modified to include an ATG start codon at the 5' end and a TAA stop codon at the 3' end. The transcriptional unit is ligated into the baculoviral transfer vector pBlueBac III (Invitrogen). The resulting transfer vector is verified by DNA sequencing. The collagen-growth factor chimera gene is transferred into the baculovirus genome (AcMNPV) by the standard in vivo recombination method. The pBlueBacIII transfer vector containing the collagen-growth factor chimera gene is cotransfected into Sf9 cells by standard methods. Recombinant viral plaques that are blue are selected and isolated by several rounds of reinfection. Pure recombinant baculovirus is verified by DNA sequencing. The recombinant virus containing the collagen-growth factor chimera gene is used to infect suspension cultures of Sf9 cells and optimal protein expression is determined at 48-72 hours post-infection. The protein product is recovered from the culture medium and analyzed by SDS-PAGE.

55 It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended hereto.

The claims which follow identify embodiments of the invention additional to those described in detail above.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: United States Surgical Corporation
 (B) STREET: 150 Glover Avenue
 (C) CITY: Norwalk
 (D) STATE: Connecticut
 (E) COUNTRY: USA
 (F) POSTAL CODE (ZIP): 06856

(ii) TITLE OF INVENTION: Recombinant chimeric proteins and methods of use thereof

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 95109019.0

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3535 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 20..3526

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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| TTCCGTGCCT GGGCCCATGG GTCCCTCTGG TCCTCGTGGT CTCCCTGGCC CCCCTGGTGC | 120 |
| ACCTGGTCCC CAAGGCTTCC AAGGTCCCCC TGGTGAGCCT GGCGAGCCTG GAGCTTCAGG | 180 |
| TCCCATGGGT CCCCAGGTC CCCCAGGTCC CCTGGAAAG AATGGAGATG ATGGGGAAGC | 240 |
| TGGAACACCT GGTGCTCCTG GTGAGCGTGG GCCTCCTGGG CTCAGGGTG CTCGAGGATT | 300 |
| GGCCGGAACA GCTGGCCTCC CTGGAATGAA GGGACACAGA GGTTCAGTG GTTTGGATGG | 360 |
| TGCCAAGGGA GATGCTGGTC CTGCTGGTCC TAAGGGTGAG CCTGGCAGCC CTGGTGAAAA | 420 |
| TGGAGCTCCT GGTGAGATGG GGGCCGTGG CCTGCCTGGT GAGAGAGGTC GCCCTGGAGC | 480 |
| CCCTGGCCCT GCTGGTGCTC GTGGAAATGA TGGTGCTACT GGTGCTGCCG GGCCCCCTGG | 540 |
| TCCACCGGC CCCGCTGGTC CTCCTGGCTT CCCTGGTGCT GTTGGTGCTA AGGGTGAAGC | 600 |

| | | | | | | | |
|----|-------------|------------|------------|------------|------------|-------------|------|
| | TGGTCCCCAA | GGGCCCCGAG | GCTCTGAAGG | TCCCCAGGGT | GTGCGTGGTG | AGCCTGGCCC | 660 |
| 5 | CCCTGGCCCT | GCTGGTGCTG | CTGGCCCTGC | TGGAACCCCT | GGTGCTGATG | GACAGCCTGG | 720 |
| | TGCTAAAGGT | GCCAATGGTG | CTCCTGGTAT | TGCTGGTGCT | CCTGGCTTCC | CTGGTGCCCCG | 780 |
| | AGGCCCTCT | GGACCCGAGG | GCCCCGGCGG | CCCTCCTGGT | CCCAAGGGTA | ACAGCGGTGA | 840 |
| 10 | ACCTGGTGCT | CCTGGCAGCA | AAGGAGACAC | TGGTGCTAAG | GGAGAGCCTG | GCCCTGTTGG | 900 |
| | TGTTCAAGGA | CCCCCTGGCC | CTGCTGGAGA | GGAAGGAAAG | CGAGGAGCTC | GAGGTGAACC | 960 |
| | CGGACCCACT | GGCCTGCCCC | GACCCCTGGG | CGAGCGTGGT | GGACCTGGTA | GCCGTGGTTT | 1020 |
| | CCCTGGCGCA | GATGGTGTTG | CTGGTCCCAA | GGGTCCCCTG | GGTGAACGTG | GTTCTCCTGG | 1080 |
| 15 | CCCCGCTGGC | CCCAAAGGAT | CTCCTGGTGA | AGCTGGTCGT | CCCGGTGAAG | CTGGTCTGCC | 1140 |
| | TGGTGCCAAG | GGTCTGACTG | GAAGCCCTGG | CAGCCCTGGT | CCTGATGGCA | AAACTGGCCC | 1200 |
| | CCCTGGTCCC | GCCGGTCAAG | ATGGTCGCCC | CGGACCCCA | GGCCACCTG | GTGCCCCTGG | 1260 |
| 20 | TCAGGCTGGT | GTGATGGGAT | TCCCTGGACC | TAAAGGTGCT | GCTGGAGAGC | CCGGCAAGGC | 1320 |
| | TGGAGAGCGA | GGTGTTCCTG | GACCCCTGGG | CGCTGTCGGT | CCTGCTGGCA | AAGATGGAGA | 1380 |
| | GGCTGGAGCT | CAGGGACCCC | CTGGCCCTGC | TGGTCCCCTG | GGCGAGAGAG | GTGAACAAGG | 1440 |
| 25 | CCCTGCTGGC | TCCCCCGGAT | TCCAGGGTCT | CCCTGGTCCT | GCTGGTCCTC | CAGGTGAAGC | 1500 |
| | AGGCAAACCT | GGTGAACAGG | GTGTTCTCTG | AGACCTTGGC | GCCCTGGCC | CCTCTGGAGC | 1560 |
| | AAGAGGCGAG | AGAGGTTTCC | CTGGCGAGCG | TGGTGTGCAA | GGTCCCCCTG | GTCTGCTGG | 1620 |
| 30 | ACCCCGAGGG | GCCAACGGTG | CTCCCGGCAA | CGATGGTGCT | AAGGGTGATG | CTGGTGCCCC | 1680 |
| | TGGAGCTCCC | GGTAGCCAGG | GCGCCCCCTG | CCTTCAGGGA | ATGCCTGGTG | AACGTGGTGC | 1740 |
| | AGCTGGTCTT | CCAGGGCCTA | AGGGTGACAG | AGGTGATGCT | GGTCCCAAAG | GTGCTGATGG | 1800 |
| 35 | CTCTCTGGC | AAAGATGGCG | TCCGTGGTCT | GACCGGCCCC | ATTGGTCCTC | CTGGCCCTGC | 1860 |
| | TGGTGCCCCCT | GGTGACAAGG | GTGAAAGTGG | TCCCAGCGGC | CCTGCTGGTC | CCACTGGAGC | 1920 |
| | TCGTGGTGCC | CCCGGAGACC | GTGGTGAGCC | TGGTCCCCCC | GGCCCTGCTG | GCTTTGCTGG | 1980 |
| 40 | CCCCCTGGT | GCTGACGGCC | AACCTGGTGC | TAAAGGCGAA | CCTGGTGATG | CTGGTGCCAA | 2040 |
| | AGGCGATGCT | GGTCCCCCTG | GGCCTGCCGG | ACCCGCTGGA | CCCCCTGGCC | CCATTGGTAA | 2100 |
| | TGTTGGTGCT | CCTGGAGCCA | AAGGTGCTCG | CGGCAGCGCT | GGTCCCCCTG | GTGCTACTGG | 2160 |
| | TTTCCCTGGT | GCTGCTGGCC | GAGTCGGTCC | TCCTGGCCCC | TCTGGAAATG | CTGGACCCCC | 2220 |
| 45 | TGGCCCTCCT | GGTCCTGCTG | GCAAAGAAGG | CGGCAAAGGT | CCCCGTGGTG | AGACTGGCCC | 2280 |
| | TGCTGGACGT | CCTGGTGAAG | TTGGTCCCCC | TGGTCCCCCT | GGCCCTGCTG | GCGAGAAAGG | 2340 |
| | ATCCCCTGGT | GCTGATGGTC | CTGCTGGTGC | TCCTGGTACT | CCCGGGCCTC | AAGGTATTGC | 2400 |
| 50 | TGGACAGCGT | GGTGTGGTCG | GCCTGCCTGG | TCAGAGAGGA | GAGAGAGGCT | TCCCTGGTCT | 2460 |
| | TCCTGGCCCC | TCTGGTGAAC | CTGGCAAACA | AGGTCCCTCT | GGAGCAAGTG | GTGAACGTGG | 2520 |
| | TCCCCCGGGT | CCCATGGGCC | CCCCTGGATT | GGCTGGACCC | CCTGGTGAAT | CTGGACGTGA | 2580 |

55

5 GGGGGCTCCT GCTGCCGAAG GTTCCCCTGG ACGAGACGGT TCTCCTGGCG CCAAGGGTGA 2640
 CCGTGGTGAG ACCGGCCCCG CTGGACCCCC TGGTGCTCNT GGTGCTCNTG GTGCCCCCTGG 2700
 CCCC GTTGGC CCTGCTGGCA AGAGTGGTGA TCCTGGTGAG ACTGGTCCTG CTGGTCCCGC 2760
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 TTTGCGACTT AGCTTCCTCC CCCAGCCACC TCAAGAGAAG GCTCACGATG GTGGCCGCTA 3180
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 25 TGTGCCCACT GAACTGAGTG CCATCTCCAT GCTGTACCTG GATGAGTATG ATAAGGTGGT 3480
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(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3541 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 20..3532

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

45 GGGGAAGGATT TCCATTTCCC AGCTGTCTTA TGGCTATGAT GAGAAATCAA CCGGAGGAAT 60
 TTCCGTGCCT GGCCCCATGG GTCCCTCTGG TCCTCGTGGT CTCCCTGGCC CCCCTGGTGC 120
 ACCTGGTCCC CAAGGCTTCC AAGGTCCCC TGGTGAGCCT GGCGAGCCTG GAGCTTCAGG 180
 50 TCCCATGGGT CCCCAGGGTC CCCCAGGTCC CCCTGGAAA AATGGAGATG ATGGGGAAGC 240
 TGGAAAACCT GGTGTCCTG GTGAGCGTGG GCCTCCTGGG CCTCAGGGTG CTCGAGGATT 300
 GCCCGGAACA GCTGGCCTCC CTGGAATGAA GGGACACAGA GGTTCAGTG GTTGGATGG 360

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|----|-------------|------------|------------|------------|------------|------------|------|
| | TGCCAAGGGA | GATGCTGGTC | CTGCTGGTCC | TAAGGGTGAG | CCTGGCAGCC | CTGGTGAAAA | 420 |
| 5 | TGGAGCTCCT | GGTCAGATGG | GCCCCCGTGG | CCTGCCTGGT | GAGAGAGGTC | GCCCTGGAGC | 480 |
| | CCCTGGCCCT | GCTGGTGCTC | GTGGAAATGA | TGGTGCTACT | GGTGCTGCCG | GGCCCCCTGG | 540 |
| | TCCCACCGGC | CCCCTGGTTC | CTCCTGGCTT | CCCTGGTGCT | GTTGGTGCTA | AGGGTGAAGC | 600 |
| 10 | TGGTCCCCAA | GGGCCCCGAG | GCTCTGAAGG | TCCCCAGGGT | GTGCGTGGTG | AGCCTGGCCC | 660 |
| | CCCTGGCCCT | GCTGGTGCTG | CTGGCCCTGC | TGGAAACCCT | GGTGCTGATG | GACAGCCTGG | 720 |
| | TGCTAAAGGT | GCCAATGGTG | CTCCTGGTAT | TGCTGGTGCT | CCTGGCTTCC | CTGGTGCCCC | 780 |
| | AGGCCCCCTCT | GGACCCAGG | GCCCCGGCGG | CCCTCCTGGT | CCCAAGGGTA | ACAGCGGTGA | 840 |
| 15 | ACCTGGTGCT | CCTGGCAGCA | AAGGAGACAC | TGGTGCTAAG | GGAGAGCCTG | GCCCTGTTGG | 900 |
| | TGTTCAAGGA | CCCCCTGGCC | CTGCTGGAGA | GGAAGGAAAG | CGAGGAGCTC | GAGGTGAACC | 960 |
| | CGGACCCACT | GGCCTGCCCG | GACCCCTGG | CGAGCGTGGT | GGACCTGGTA | GCCGTGGTTT | 1020 |
| 20 | CCCTGGCGCA | GATGGTGTG | CTGGTCCCAA | GGGTCCCCTG | GGTGAACGTG | GTTCTCCTGG | 1080 |
| | CCCCGCTGGC | CCCAAAGGAT | CTCCTGGTGA | AGCTGGTCGT | CCCGGTGAAG | CTGGTCTGCC | 1140 |
| | TGGTGCCAAG | GGTCTGACTG | GAAGCCCTGG | CAGCCCTGGT | CCTGATGGCA | AAACTGGCCC | 1200 |
| 25 | CCCTGGTCCC | GCCGGTCAAG | ATGGTCGCCC | CGGACCCCCA | GGCCACCTG | GTGCCGTGG | 1260 |
| | TCAGGCTGGT | GTGATGGGAT | TCCCTGGACC | TAAAGGTGCT | GCTGGAGAGC | CCGGCAAGGC | 1320 |
| | TGGAGAGCGA | GGTGTTCCTG | GACCCCTGG | CGCTGTCGGT | CCTGCTGGCA | AAGATGGAGA | 1380 |
| 30 | GGCTGGAGCT | CAGGGACCCC | CTGGCCCTGC | TGGTCCCCTG | GGCGAGAGAG | GTGAACAAGG | 1440 |
| | CCCTGCTGGC | TCCCCCGGAT | TCCAGGGTCT | CCCTGGTCCT | GCTGGTCCTC | CAGGTGAAGC | 1500 |
| | AGGCAAACCT | GGTGAACAGG | GTGTTCTCTG | AGACCTTGGC | GCCCCCTGGC | CCTCTGGAGC | 1560 |
| 35 | AAGAGGCGAG | AGAGGTTTCC | CTGGCGAGCG | TGGTGCGCAA | GGTCCCCCTG | GTCCTGCTGG | 1620 |
| | ACCCCGAGGG | GCCAACGGTG | CTCCCGGCAA | CGATGGTGCT | AAGGGTGATG | CTGGTGCCCC | 1680 |
| | TGGAGCTCCC | GGTAGCCAGG | GCGCCCCTGG | CCTTCAGGGA | ATGCCTGGTG | AACGTGGTGC | 1740 |
| 40 | AGCTGGTCTT | CCAGGGCCTA | AGGGTGACAG | AGGTGATGCT | GGTCCCAAAG | GTGCTGATGG | 1800 |
| | CTCTCCTGGC | AAAGATGGCG | TCCGTGGTCT | GACCGGCCCC | ATTGGTCCTC | CTGGCCCTGC | 1860 |
| | TGGTGCCCCCT | GGTGACAAGG | GTGAAAGTGG | TCCCAGCGGC | CCTGCTGGTC | CCACTGGAGC | 1920 |
| | TCGTGGTGCC | CCCGGAGACC | GTGGTGAGCC | TGGTCCCCCC | GGCCCTGCTG | GCTTTGCTGG | 1980 |
| 45 | CCCCCTGGT | GCTGACGGCC | AACCTGGTGC | TAAAGGCGAA | CCTGGTGATG | CTGGTGCCAA | 2040 |
| | AGGCGATGCT | GGTCCCCCTG | GGCCTGCCGG | ACCCGCTGGA | CCCCCTGGCC | CCATTGGTAA | 2100 |
| | TGTTGGTGCT | CCTGGAGCCA | AAGGTGCTCG | CGGCAGCGCT | GGTCCCCCTG | GTGCTACTGG | 2160 |
| 50 | TTTCCCTGGT | GCTGCTGGCC | GAGTCGGTCC | TCCTGGCCCC | TCTGGAAATG | CTGGACCCCC | 2220 |
| | TGCCCCCTCT | GGTCCTGCTG | GCAAAGAAGG | CGGCAAAGGT | CCCCGTGGTG | AGACTGGCCC | 2280 |
| | TGCTGGACGT | CCTGGTGAAG | TTGGTCCCCC | TGGTCCCCCT | GGCCCTGCTG | GCGAGAAAGG | 2340 |

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ATCCCCTGGT GCTGATGGTC CTGCTGGTGC TCCTGGTACT CCCGGGCCTC AAGGTATTGC 2400
 5 TGGACAGCGT GGTGTGGTCG GCCTGCCTGG TCAGAGAGGA GAGAGAGGCT TCCCTGGTCT 2460
 TCCTGGCCCC TCTGGTGAAC CTGGCAAACA AGGTCCCTCT GGAGCAAGTG GTGAACGTGG 2520
 TCCCCCGGT CCCATGGGCC CCCCTGGATT GGCTGGACCC CCTGGTGAAT CTGGACGTGA 2580
 10 GGGGGCTCCT GCTGCCGAAG GTTCCCTTGG ACGAGACGGT TCTCCTGGCG CCAAGGGTGA 2640
 CCGTGGTGAG ACCGGCCCCG CTGGACCCCC TGGTGCTCNT GGTGCTCNTG GTGCCCTGG 2700
 CCCCCTGGC CCTGTGGCA AGAGTGGTGA TCGTGGTGAG ACTGGTCTG CTGGTCCCGC 2760
 CGGTCCCGTC GGGCCCGCTG GCGCCCGTGG CCCC GCCGA CCCCAAGGCC CCCGTGGTGA 2820
 15 CAAGGGTGAG ACAGCGCAAC AGGGCGACAG AGGCATAAAG GGTCAACCGTG GCTTCTCTGG 2880
 CCTCCAGGT CCCCCTGGCC CTCCTGGCTC TCCTGGTGAA CAAGGTCCCT CTGGAGCCTC 2940
 TGGTCTGCT GGTCCCCGAG GTCCCCCTGG CTCTGCTGGT GTCCTGGCA AAGATGGACT 3000
 20 CAACGGTCTC CTGGCCCCA TTGGCCCCC TGGTCTCGC GGTGCGACTG GTGATGCTGG 3060
 TCCTGTTGGT CCCCCCGGCC CTCCTGGACC TCCTGGTCCC CCTGGTCTC CCAGCGCTGG 3120
 TTTCGACTTC AGCTTCTCTC CCCAGCCACC TCAAGAGAAG GCTCACGATG GTGGCCGCTA 3180
 25 CTACCGGGCT AGATCTGCCC TGGACACCAA CTATTGCTTC AGCTCCACGG AGAAGAACTG 3240
 CTGCGTGCGG CAGCTGTACA TTGACTTCG CAAGGACCTC GGCTGGAAGT GGATCCACGA 3300
 GCCCAAGGGC TACCATGCCA ACTTCTGCCT CGGGCCCTGC CCCTACATTG GGAGCCTGGA 3360
 30 CACGCAGTAC AGCAAGGTCC TGGCCCTGTA CAACCAGCAT AACC CGGCG CCTCGGCGGC 3420
 GCCGTGCTGC GTGCCGAGG CGCTGGAGCC GCTGCCCATC GTGTACTACG TGGGCCGCAA 3480
 GCCCAAGGTG GAGCAGCTGT CCAACATGAT CGTGCGCTCC TGCAAGTGCA GCTGATCTAG 3540
 35 A 3541

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4192 base pairs
 (B) TYPE: nucleic acid
 40 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 20..4183

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGGGAAGGATT TCCATTTCCC AGCTGTCTTA TGGCTATGAT GAGAAATCAA CCGGAGGAAT 60
 TTCCGTGCCT GGCCCCATGG GTCCCTCTGG TCCTCGTGGT CTCCTGGCC CCCCTGGTGC 120

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|----|--|------|
| | ACCTGGTCCC CAAGGCTTCC AAGGTCCCCC TGGTGAGCCT GCGGAGCCTG GAGCTTCAGG | 180 |
| 5 | TCCCATGGGT CCCCAGAGTC CCCCAGGTCC CCCTGGAAAG AATGGAGATG ATGGGGAAAGC | 240 |
| | TGGAAAACCT GGTGCTCCTG GTGAGCGTGG GCCTCCTGGG CCTCAGGGTG CTCGAGGATT | 300 |
| | GCCCCGAACA GCTGGCCTCC CTGGAATGAA GGGACACAGA GGTTCAGTG GTTTGGATGG | 360 |
| 10 | TGCCAAGGGA GATGCTGGTC CTGCTGGTCC TAAGGGTGAG CCTGGCAGCC CTGGTGAAAA | 420 |
| | TGGAGCTCCT GGTGAGATGG GCCCCCGTGG CCTGCCTGGT GAGAGAGGTC GCCCTGGAGC | 480 |
| | CCCTGGCCCT GCTGGTGCTC GTGGAAATGA TGGTGCTACT GGTGCTGCCG GGGCCCTGG | 540 |
| | TCCCACCGGC CCCGCTGGTC CTCCTGGCTT CCCTGGTGCT GTTGGTGCTA AGGGTGAAGC | 600 |
| 15 | TGGTCCCCAA GGGCCCCGAG GCTCTGAAGG TCCCCAGGGT GTGCGTGGTG AGCCTGGCCC | 660 |
| | CCCTGGCCCT GCTGGTGCTG CTGGCCCTGC TGGAAACCCT GGTGCTGATG GACAGCCTGG | 720 |
| | TGCTAAAGGT GCCAATGGTG CTCCTGGTAT TGCTGGTGCT CCTGGCTTCC CTGGTGCCCCG | 780 |
| 20 | AGGCCCTCTT GGACCCAGG GCCCCGCGG CCCTCCTGGT CCAAGGGTA ACAGCGGTGA | 840 |
| | ACCTGGTGCT CCTGGCAGCA AAGGAGACAC TGGTGCTAAG GGAGAGCCTG GCCCTGTTGG | 900 |
| | TGTTCAAGGA CCCCCTGGCC CTGCTGGAGA GGAAGGAAAG CGAGGAGCTC GAGGTGAACC | 960 |
| 25 | CGGACCCACT GGCCTGCCCG GACCCCTGG CGAGCGTGGT GGACCTGGTA GCCGTGGTTT | 1020 |
| | CCCTGGCGCA GATGGTGTTG CTGGTCCCAA GGGTCCCGCT GGTGAACGTG GTTCTCCTGG | 1080 |
| | CCCCGCTGGC CCCAAAGGAT CTCCTGGTGA AGCTGGTCGT CCCGGTGAAG CTGGTCTGCC | 1140 |
| 30 | TGGTGCCAAG GGTCTGACTG GAAGCCCTGG CAGCCCTGGT CCTGATGGCA AAACCTGGCCC | 1200 |
| | CCCTGGTCCC GCCGGTCAAG ATGGTCGCCC CGGACCCCCA GGGCCACCTG GTGCCCGTGG | 1260 |
| | TCAGGCTGGT GTGATGGGAT TCCCTGGACC TAAAGGTGCT GCTGGAGAGC CCGGCAAGGC | 1320 |
| 35 | TGGAGAGCGA GGTGTTCCCG GACCCCTGG CGCTGTCGGT CCTGCTGGCA AAGATGGAGA | 1380 |
| | GGCTGGAGCT CAGGGACCCC CTGGCCCTGC TGGTCCCGCT GCGGAGAGAG GTGAACAAGG | 1440 |
| | CCCTGCTGGC TCCCCCGGAT TCCAGGTCT CCCTGGTCCT GCTGGTCCTC CAGGTGAAGC | 1500 |
| | AGGCAACCTT GGTGAACAGG GTGTTCTTGG AGACCTTGGC GGGCCTGGCC CCTCTGGAGC | 1560 |
| 40 | AAGAGGCGAG AGAGGTTTCC CTGGCGAGCG TGGTGTCGAA GGTCCCCCTG GTCCTGCTGG | 1620 |
| | ACCCCGAGGG GCCAACGGTG CTCCCGGCAA CGATGGTGCT AAGGGTGATG CTGGTGCCCC | 1680 |
| | TGGAGCTCCC GGTAGCCAGG GCGCCCTGG CCTTCAGGGA ATGCCTGGTG AACGTGGTGC | 1740 |
| 45 | AGCTGGTCTT CCAGGGCCTA AGGGTGACAG AGGTGATGCT GGTCCCAAAG GTGCTGATGG | 1800 |
| | CTCTCCTGGC AAAGATGGCG TCCGTGGTCT GACCGGCCCC ATTGGTCCTC CTGGCCCTGC | 1860 |
| | TGGTGCCCCCT GGTGACAAGG GTGAAAGTGG TCCCAGCGGC CCTGCTGGTC CCACTGGAGC | 1920 |
| 50 | TCGTGGTGCC CCCGGAGACC GTGGTGAGCC TGGTCCCCC GGGCCTGCTG GCTTTGCTGG | 1980 |
| | CCCCCTGGT GCTGACGGCC AACCTGGTGC TAAAGGCGAA CCTGGTGATG CTGGTGCCAA | 2040 |
| | AGGCGATGCT GGTCCCCCTG GGCCTGCCGG ACCCGCTGGA CCCCCTGGCC CCATTGGTAA | 2100 |

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|----|---|------|
| | TGTTGGTGCT CCTGGAGCCA AAGGTGCTCG CGGCAGCGCT GGTCCCCCTG GTGCTACTGG | 2160 |
| 5 | TTTCCTTGGT GCTGCTGGCC GAGTCGGTCC TCCTGGCCCC TCTGGAAATG CTGGACCCCC | 2220 |
| | TGGCCCTCCT GGTCTGTCTG GCAAAGAAGG CGGCAAAGGT CCCCCTGGTG AGACTGGCCC | 2280 |
| | TGCTGGACGT CCTGGTGAAG TTGGTCCCCC TGGTCCCCCT GGCCCTGCTG GCGAGAAAGG | 2340 |
| 10 | ATCCCCTGGT GCTGATGGTC CTGCTGGTGC TCCTGGTACT CCCGGGCCTC AAGGTATTGC | 2400 |
| | TGGACAGCGT GGTGTGGTCG GCCTGCCTGG TCAGAGAGGA GAGAGAGGCT TCCCTGGTCT | 2460 |
| | TCCTGGCCCC TCTGGTGAAC CTGGCAAACA AGGTCCCTCT GGAGCAAAGT GTGAACGTGG | 2520 |
| 15 | TCCCCCGGT CCCATGGGCC CCCCTGGATT GGCTGGACCC CCTGGTGAAT CTGGACGTGA | 2580 |
| | GGGGGCTCCT GCTGCCGAAG GTTCCCCTGG ACGAGACGGT TCTCCTGGCG CCAAGGGTGA | 2640 |
| | CCGTGGTGAG ACCGGCCCCG CTGGACCCCC TGGTGTCTNT GGTGTCTNTG GTGCCCTGG | 2700 |
| 20 | CCCCGTTGGC CTGCTGGCA AGAGTGGTGA TCGTGGTGAG ACTGGTCCTG CTGGTCCCGC | 2760 |
| | CGGTCCCCTC GGGCCCCGTG GCGCCCGTGG CCCC GCCGGA CCCC AAGGCC CCCGTGGTGA | 2820 |
| | CAAGGGTGAG ACAGGCCAAC AGGGCGACAG AGGCATAAAG GGTCAACGTG GCTTCTCTGG | 2880 |
| | CCTCCAGGGT CCCCTGGGCC CTCCTGGCTC TCCTGGTGAA CAAGGTCCCT CTGGAGCCTC | 2940 |
| 25 | TGGTCTGCT GGTCCCCGAG GTCCCCCTGG CTCTGCTGGT GCTCCTGGCA AAGATGGACT | 3000 |
| | CAACGGTCTC CTGGCCCCA TTGGGCCCCC TGGTCTCGC GGTGCACTG GTGATGCTGG | 3060 |
| | TCCTGTTGGT CCCCCCGGCC CTCCTGGACC TCCTGGTCCC CCTGGTCCTC CCAGCGCTGG | 3120 |
| 30 | TTTCGACTTC AGCTTCCTCC CCCAGCCACC TCAAGAGAAG GCTCACGATG GTGGCCGCTA | 3180 |
| | CTACCGGGCT AGATCTGATG AGGCTTCTGG GATAGGCCCA GAAGTTCCTG ATGACCGCGA | 3240 |
| | CTTCGAGCCC TCCCTAGGCC CAGTGTGCCC CTTCGCTGT CAATGCCATC TTCGAGTGGT | 3300 |
| 35 | CCAGTGTCTT GATTGGGGT TGGACAAAGT GCCAAAGGAT CTTCCCCCTG ACACAACCTT | 3360 |
| | GCTAGACCTG CAAAACAACA AAATAACCGA AATCAAAGAT GGAGACTTTA AGAACCTGAA | 3420 |
| | GAACCTTCAC GCATTGATTG TTGTCAACAA TAAATTAGC AAAGTTAGTC CTGGAGCATT | 3480 |
| 40 | TACACCTTTG GTGAAGTTGG AACGACTTTA TCTGTCCAAG AATCAGCTGA AGGAATTGCC | 3540 |
| | AGAAAAATG CCCAAACTC TTCAGGAGCT GCGTGCCCAT GAGAATGAGA TCACCAAAGT | 3600 |
| | GCGAAAAGTT ACTTTCAATG GACTGAACCA GATGATTGTC ATAGAAGTGG GCACCAATCC | 3660 |
| 45 | GCTGAAGAGC TCAGGAATTG AAAATGGGGC TTTCCAGGGA ATGAAGAAGC TCTCCTACAT | 3720 |
| | CCGCATTGCT GATACCAATA TCACCAGCAT TCCTCAAGGT CTTCTCTCTT CCCTTACGGA | 3780 |
| | ATTACATCTT GATGGCAACA AAATCAGCAG AGTTGATGCA GCTAGCCTGA AAGGACTGAA | 3840 |
| | TAATTTGGCT AAGTTGGGAT TGAGTTTCAA CAGCATCTCT GCTGTTGACA ATGGCTCTCT | 3900 |
| 50 | GGCCAACACG CCTCATCTGA GGGAGCTTCA CTGGACAAC AACAAGCTTA CCAGAGTACC | 3960 |
| | TGGTGGGCTG GCAGAGCATA AGTACATCCA GGTGTCTAC CTCATAACA ACAATATCTC | 4020 |
| | TGTAGTTGGA TCAAGTGACT TCTGCCACC TGGACACAAC ACCAAAAAGG CTTCTTATTC | 4080 |

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GGGTGTGAGT CTTTTCAGCA ACCCGGTCCA GTACTGGGAG ATACAGCCAT CCACCTTCAG 4140
 5 ATGTGTCTAC GTGCGCTCTG CCATTCAACT CGGAACTAT AAGTAACTGC AG 4192

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3349 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 20..3340

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGAAGGATT TCCATTTCCC AGCTGTCTTA TGCTATGAT GAGAAATCAA CCGGAGGAAT 60
 TTCCGTGCCT GGCCCCATGG GTCCCTCTGG TCCTCGTGGT CTCCTGGCC CCCCTGGTGC 120
 ACCTGGTCCC CAAGGCTTCC AAGGTCCCC TGGTGAGCCT GCGAGCCTG GAGCTTCAGG 180
 25 TCCCATGGGT CCCCAGGTC CCCAGGTCC CCCTGGAAAG AATGGAGATG ATGGGGAAGC 240
 TGGAAAACCT GGTCTCTCTG GTGAGCGTGG GCCTCCTGGG CCTCAGGGTG CTCGAGGATT 300
 GCCCGGAACA GCTGGCCTCC CTGGAATGAA GGGACACAGA GGTTCAGTG GTTTGGATGG 360
 30 TGCCAAGGGA GATGCTGGTC CTGCTGGTCC TAAGGGTGAG CCTGGCAGCC CTGGTGAAAA 420
 TGGAGCTCCT GGTCTAGATGG GCCCCCGTGG CCTGCCTGGT GAGAGAGGTC GCCCTGGAGC 480
 CCCTGGCCCT GCTGGTGCTC GTGGAATGA TGGTGCTACT GGTGCTGCCG GGCCCCCTGG 540
 35 TCCCACCGGC CCGCTGGTC CTCCTGGCTT CCCTGGTGCT GTTGGTGCTA AGGGTGAAGC 600
 TGGTCCCCAA GGGCCCCGAG GCTCTGAAGG TCCCCAGGGT GTGCGTGGTG AGCCTGGCCC 660
 CCCTGGCCCT GCTGGTGCTG CTGGCCCTGC TGGAAACCTT GGTGCTGATG GACAGCCTGG 720
 40 TGCTAAAGGT GCCAATGGTG CTCCTGGTAT TGCTGGTGCT CCTGGCTTCC CTGGTGCCCC 780
 AGGCCCCCTCT GGACCCGAGG GCCCCGGCGG CCCTCCTGGT CCCAAGGGTA ACAGCGGTGA 840
 ACCTGGTGCT CCTGGCAGCA AAGGAGACAC TGGTGCTAAG GGAGAGCCTG GCCCTGTTGG 900
 45 TGTTCAAGGA CCCCTGGCC CTGCTGGAGA GGAAGGAAAG CGAGGAGCTC GAGGTGAACC 960
 CGGACCCACT GGCTGCCCC GACCCCTGG CGAGCGTGGT GGACCTGGTA GCCGTGGTTT 1020
 CCCTGGCGCA GATGGTGTG CTGGTCCCA GGGTCCCGT GGTGAACGTG GTTCTCCTGG 1080
 CCCCCTGGC CCCAAAGGAT CTCCTGGTGA AGCTGGTCGT CCCGGTGAAG CTGGTCTGCC 1140
 50 TGGTGCCAAG GGTCTGACTG GAAGCCCTGG CAGCCCTGGT CCTGATGGCA AAAGTGGCCC 1200
 CCCTGGTCCC GCCGGTCAAG ATGGTCGCCC CGGACCCCA GGCCACCTG GTGCCCCTGG 1260
 TCAGGCTGGT GTGATGGGAT TCCCTGGACC TAAAGGTGCT GCTGGAGAGC CCGGCAAGGC 1320

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| | TGGAGAGCGA GGTGTTCCCG GACCCCTGG CGTGTCGGT CCTGCTGGCA AAGATGGAGA | 1380 |
| 5 | GGCTGGAGCT CAGGGACCCC CTGGCCCTGC TGGTCCCGCT GGCGAGAGAG GTGAACAAGG | 1440 |
| | CCCTGCTGGC TCCCCCGGAT TCCAGGGTCT CCCTGGTCCT GCTGGTCCTC CAGGTGAAGC | 1500 |
| | AGGCAAACCT GGTGAACAGG GTGTTCTTGG AGACCTTGGC GCCCTGGCC CCTCTGGAGC | 1560 |
| 10 | AAGAGGCGAG AGAGGTTTCC CTGGCGAGCG TGGTGTGCAA GGTCCTCCCTG GTCCTGCTGG | 1620 |
| | ACCCCGAGGG GCCAACGGTG CTCCCGGCAA CGATGGTGCT AAGGGTGATG CTGGTGCCCC | 1680 |
| | TGGAGCTCCC GGTAGCCAGG GCGCCCTGG CCTTCAGGGA ATGCCTGGTG AACGTGGTGC | 1740 |
| 15 | AGCTGGTCTT CCAGGGCCTA AGGGTGACAG AGGTGATGCT GTCCCAAAG GTGCTGATGG | 1800 |
| | CTCTCCTGGC AAAGATGGCG TCCGTGGTCT GACCGGCCCC ATTGGTCCTC CTGGCCCTGC | 1860 |
| | TGGTGCCCTT GGTGACAAGG GTGAAAGTGG TCCCAGCGGC CCTGCTGGTC CCACTGGAGC | 1920 |
| 20 | TCGTGGTGCC CCCGGAGACC GTGGTGAGCC TGGTCCCCC GGCCCTGCTG GCTTTGCTGG | 1980 |
| | CCCCCTGGT GCTGACGGCC AACCTGGTGC TAAAGGCGAA CCTGGTGATG CTGGTGCCAA | 2040 |
| | AGGCGATGCT GGTCCCCCTG GGCTGCGCG ACCCGCTGGA CCCCCTGGCC CCATTGGTAA | 2100 |
| | TGTTGGTGCT CCTGGAGCCA AAGGTGCTCG CGGCAGCGCT GGTCCCCCTG GTGCTACTGG | 2160 |
| 25 | TTTCCCTGGT GCTGCTGGCC GAGTCGGTCC TCCTGGCCCC TCTGAAATG CTGGACCCCC | 2220 |
| | TGGCCCTCCT GGTCTGCTG GCAAAGAAG CGGCAAAGGT CCCCCTGGTG AGACTGGCCC | 2280 |
| | TGCTGGACGT CCTGGTGAAG TTGGTCCCC TGGTCCCCCT GGCCCTGCTG GCGAGAAAGG | 2340 |
| 30 | ATCCCCTGGT GCTGATGGTC CTGCTGGTGC TCCTGGTACT CCCGGGCCCTC AAGGTATTGC | 2400 |
| | TGGACAGCGT GGTGTGGTCG GCCTGCCTGG TCAGAGAGGA GAGAGAGGCT TCCCTGGTCT | 2460 |
| | TCCTGGCCCC TCTGGTGAAC CTGGCAAACA AGGTCCCTCT GGAGCAAGTG GTGAACGTGG | 2520 |
| 35 | TCCCCCGGT CCCATGGGCC CCCCTGGATT GGCTGGACCC CCTGGTGAAT CTGGACGTGA | 2580 |
| | GGGGGCTCCT GCTGCCGAAG GTTCCCTTGG ACGAGACGGT TCTCCTGGCG CCAAGGGTGA | 2640 |
| | CCGTGCTGAG ACCGGCCCCG CTGGACCCCC TGGTGCTCNT GGTGCTCNTG GTGCCCTGG | 2700 |
| 40 | CCCCGTTGGC CCTGCTGGCA AGAGTGGTGA TCGTGGTGAG ACTGGTCCTG CTGGTCCCGC | 2760 |
| | CGGTCCCGTC GGCCCGCTG GCGCCCGTGG CCCC GCCGA CCCCAGGCC CCCGTGGTGA | 2820 |
| | CAAGGGTGAG ACAGGCGAAC AGGGCGACAG AGGCATAAAG GGTACCGTG GCTTCTCTGG | 2880 |
| 45 | CCTCCAGGGT CCCCCTGGCC CTCCTGGCTC TCCTGGTGAA CAAGGTCCCT CTGGAGCCTC | 2940 |
| | TGGTCTGCT GGTCCCCGAG GTCCCCCTGG CTCTGCTGGT GCTCCTGGCA AAGATGGAAT | 3000 |
| | CAACGGTCTC CCTGGCCCCA TTGGGCCCCC TGGTCTCGC GGTGCACTG GTGATGCTGG | 3060 |
| | TCCTGTTGGT CCCCCCGGCC CTCCTGGACC TCCTGGTCCC CCTGGTCCTC CCAGCGCTGG | 3120 |
| 50 | TTTCGACTTC AGCTTCTCTC CCCAGCCACC TCAAGAGAAG GCTCAGATG GTGGCCGCTA | 3180 |
| | CTACCGGGCT AGATCTCCAA AGGATCTTCC CCCTGACACA ACTCTGCTAG ACCTGCAAAA | 3240 |
| 55 | CAACAAAATA ACCGAAATCA AAGATGGAGA CTTTAAGAAC CTGAAGAACC TTCACGCATT | 3300 |

GATTCTTGTC AACATAAAAA TTAGCAAAGT TAGTCCTGGA TAACTGCAG

3349

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1169 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Gln Leu Ser Tyr Gly Tyr Asp Glu Lys Ser Thr Gly Gly Ile Ser Val
 1 5 10 15
 Pro Gly Pro Met Gly Pro Ser Gly Pro Arg Gly Leu Pro Gly Pro Pro
 20 25 30
 Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Pro Pro Gly Glu Pro Gly
 35 40 45
 Glu Pro Gly Ala Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro
 50 55 60
 Pro Gly Lys Asn Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Arg Pro
 65 70 75 80
 Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Leu Pro Gly
 85 90 95
 Thr Ala Gly Leu Pro Gly Met Lys Gly His Arg Gly Phe Ser Gly Leu
 100 105 110
 Asp Gly Ala Lys Gly Asp Ala Gly Pro Ala Gly Pro Lys Gly Glu Pro
 115 120 125
 Gly Ser Pro Gly Glu Asn Gly Ala Pro Gly Gln Met Gly Pro Arg Gly
 130 135 140
 Leu Pro Gly Glu Arg Gly Arg Pro Gly Ala Pro Gly Pro Ala Gly Ala
 145 150 155 160
 Arg Gly Asn Asp Gly Ala Thr Gly Ala Ala Gly Pro Pro Gly Pro Thr
 165 170 175
 Gly Pro Ala Gly Pro Pro Gly Phe Pro Gly Ala Val Gly Ala Lys Gly
 180 185 190
 Glu Ala Gly Pro Gln Gly Pro Arg Gly Ser Glu Gly Pro Gln Gly Val
 195 200 205
 Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Ala Ala Gly Pro Ala
 210 215 220
 Gly Asn Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Ala Asn Gly
 225 230 235 240
 Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Ala Arg Gly Pro
 245 250 255
 Ser Gly Pro Gln Gly Pro Gly Gly Pro Pro Gly Pro Lys Gly Asn Ser
 260 265 270
 Gly Glu Pro Gly Ala Pro Gly Ser Lys Gly Asp Thr Gly Ala Lys Gly

| | 275 | 280 | 285 |
|----|--|--|-----|
| 5 | Glu Pro Gly Pro Val Gly Val 290 295 | Gln Gly Pro Pro Gly Pro Ala Gly Glu 300 | |
| | Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Pro Thr Gly Leu Pro 305 310 315 320 | | |
| 10 | Gly Pro Pro Gly Glu Arg Gly Gly Pro Gly Ser Arg Gly Phe Pro Gly 325 330 335 | | |
| | Ala Asp Gly Val Ala Gly Pro Lys Gly Pro Ala Gly Glu Arg Gly Ser 340 345 350 | | |
| 15 | Pro Gly Pro Ala Gly Pro Lys Gly Ser Pro Gly Glu Ala Gly Arg Pro 355 360 365 | | |
| | Gly Glu Ala Gly Leu Pro Gly Ala Lys Gly Leu Thr Gly Ser Pro Gly 370 375 380 | | |
| 20 | Ser Pro Gly Pro Asp Gly Lys Thr Gly Pro Pro Gly Pro Ala Gly Gln 385 390 395 400 | | |
| | Asp Gly Arg Pro Gly Pro Pro Gly Pro Pro Gly Ala Arg Gly Gln Ala 405 410 415 | | |
| 25 | Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Ala Gly Glu Pro Gly 420 425 430 | | |
| | Lys Ala Gly Glu Arg Gly Val Pro Gly Pro Pro Gly Ala Val Gly Pro 435 440 445 | | |
| 30 | Ala Gly Lys Asp Gly Glu Ala Gly Ala Gln Gly Pro Pro Gly Pro Ala 450 455 460 | | |
| | Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Pro Ala Gly Ser Pro Gly 465 470 475 480 | | |
| 35 | Phe Gln Gly Leu Pro Gly Pro Ala Gly Pro Pro Gly Glu Ala Gly Lys 485 490 495 | | |
| | Pro Gly Glu Gln Gly Val Pro Gly Asp Leu Gly Ala Pro Gly Pro Ser 500 505 510 | | |
| 40 | Gly Ala Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Val Gln Gly 515 520 525 | | |
| | Pro Pro Gly Pro Ala Gly Pro Arg Gly Ala Asn Gly Ala Pro Gly Asn 530 535 540 | | |
| 45 | Asp Gly Ala Lys Gly Asp Ala Gly Ala Pro Gly Ala Pro Gly Ser Gln 545 550 555 560 | | |
| | Gly Ala Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala Gly 565 570 575 | | |
| 50 | Leu Pro Gly Pro Lys Gly Asp Arg Gly Asp Ala Gly Pro Lys Gly Ala 580 585 590 | | |
| | Asp Gly Ser Pro Gly Lys Asp Gly Val Arg Gly Leu Thr Gly Pro Ile 595 600 605 | | |
| 55 | Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Asp Lys Gly Glu Ser Gly 610 615 620 | | |
| | Pro Ser Gly Pro Ala Gly Pro Thr Gly Ala Arg Gly Ala Pro Gly Asp | | |

| | | | | | | | |
|----|---|---|-----|-----|-----|-----|-----|
| | 625 | | 630 | | 635 | | 640 |
| 5 | Arg Gly Glu Pro Gly | Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro Pro | | | | | |
| | | 645 | | 650 | | 655 | |
| | Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Pro Gly Asp Ala Gly | | | | | | |
| | | 660 | | 665 | | 670 | |
| 10 | Ala Lys Gly Asp Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Pro | | | | | | |
| | | 675 | | 680 | | 685 | |
| | Pro Gly Pro Ile Gly Asn Val Gly Ala Pro Gly Ala Lys Gly Ala Arg | | | | | | |
| | | 690 | | 695 | | 700 | |
| 15 | Gly Ser Ala Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly | | | | | | |
| | | 705 | | 710 | | 715 | |
| | Arg Val Gly Pro Pro Gly Pro Ser Gly Asn Ala Gly Pro Pro Gly Pro | | | | | | |
| | | 725 | | 730 | | 735 | |
| 20 | Pro Gly Pro Ala Gly Lys Glu Gly Gly Lys Gly Pro Arg Gly Glu Thr | | | | | | |
| | | 740 | | 745 | | 750 | |
| | Gly Pro Ala Gly Arg Pro Gly Glu Val Gly Pro Pro Gly Pro Pro Gly | | | | | | |
| | | 755 | | 760 | | 765 | |
| | Pro Ala Gly Glu Lys Gly Ser Pro Gly Ala Asp Gly Pro Ala Gly Ala | | | | | | |
| | | 770 | | 775 | | 780 | |
| 25 | Pro Gly Thr Pro Gly Pro Gln Gly Ile Ala Gly Gln Arg Gly Val Val | | | | | | |
| | | 785 | | 790 | | 795 | |
| | Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly | | | | | | |
| | | 805 | | 810 | | 815 | |
| 30 | Pro Ser Gly Glu Pro Gly Lys Gln Gly Pro Ser Gly Ala Ser Gly Glu | | | | | | |
| | | 820 | | 825 | | 830 | |
| | Arg Gly Pro Pro Gly Pro Met Gly Pro Pro Gly Leu Ala Gly Pro Pro | | | | | | |
| | | 835 | | 840 | | 845 | |
| 35 | Gly Glu Ser Gly Arg Glu Gly Ala Pro Ala Ala Glu Gly Ser Pro Gly | | | | | | |
| | | 850 | | 855 | | 860 | |
| | Arg Asp Gly Ser Pro Gly Ala Lys Gly Asp Arg Gly Glu Thr Gly Pro | | | | | | |
| | | 865 | | 870 | | 875 | |
| 40 | Ala Gly Pro Pro Gly Ala Xaa Gly Ala Xaa Gly Ala Pro Gly Pro Val | | | | | | |
| | | 885 | | 890 | | 895 | |
| | Gly Pro Ala Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro Ala Gly | | | | | | |
| | | 900 | | 905 | | 910 | |
| 45 | Pro Ala Gly Pro Val Gly Pro Ala Gly Ala Arg Gly Pro Ala Gly Pro | | | | | | |
| | | 915 | | 920 | | 925 | |
| | Gln Gly Pro Arg Gly Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg | | | | | | |
| | | 930 | | 935 | | 940 | |
| | Gly Ile Lys Gly His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly | | | | | | |
| | | 945 | | 950 | | 955 | |
| 50 | Pro Pro Gly Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro | | | | | | |
| | | 965 | | 970 | | 975 | |
| 55 | Ala Gly Pro Arg Gly Pro Pro Gly Ser Ala Gly Ala Pro Gly Lys Asp | | | | | | |

5 980 985 990

Gly Leu Asn Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly
995 1000 1005

Arg Thr Gly Asp Ala Gly Pro Val Gly Pro Pro Gly Pro Pro Gly Pro
1010 1015 1020

10 Pro Gly Pro Pro Gly Pro Pro Ser Ala Gly Phe Asp Phe Ser Phe Leu
1025 1030 1035 1040

Pro Gln Pro Pro Gln Glu Lys Ala His Asp Gly Gly Arg Tyr Tyr Arg
1045 1050 1055

15 Ala Arg Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg Arg His
1060 1065 1070

Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
1075 1080 1085

20 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe
1090 1095 1100

Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr
1105 1110 1115 1120

Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro
1125 1130 1135

25 Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr Asp Lys
1140 1145 1150

Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly Cys Gly Cys
1155 1160 1165

30 Arg

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1171 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

40 Gln Leu Ser Tyr Gly Tyr Asp Glu Lys Ser Thr Gly Gly Ile Ser Val
1 5 10 15

Pro Gly Pro Met Gly Pro Ser Gly Pro Arg Gly Leu Pro Gly Pro Pro
20 25 30

45 Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Pro Pro Gly Glu Pro Gly
35 40 45

Glu Pro Gly Ala Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro
50 55 60

50 Pro Gly Lys Asn Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Arg Pro
65 70 75 80

Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Leu Pro Gly
85 90 95

55

5 Thr Ala Gly Leu Pro Gly Met Lys Gly His Arg Gly Phe Ser Gly Leu
 100 105 110
 Asp Gly Ala Lys Gly Asp Ala Gly Pro Ala Gly Pro Lys Gly Glu Pro
 115 120 125
 Gly Ser Pro Gly Glu Asn Gly Ala Pro Gly Gln Met Gly Pro Arg Gly
 130 135 140
 10 Leu Pro Gly Glu Arg Gly Arg Pro Gly Ala Pro Gly Pro Ala Gly Ala
 145 150 155 160
 Arg Gly Asn Asp Gly Ala Thr Gly Ala Ala Gly Pro Pro Gly Pro Thr
 165 170 175
 15 Gly Pro Ala Gly Pro Pro Gly Phe Pro Gly Ala Val Gly Ala Lys Gly
 180 185 190
 Glu Ala Gly Pro Gln Gly Pro Arg Gly Ser Glu Gly Pro Gln Gly Val
 195 200 205
 20 Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Ala Ala Gly Pro Ala
 210 215 220
 Gly Asn Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Ala Asn Gly
 225 230 235 240
 25 Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Ala Arg Gly Pro
 245 250 255
 Ser Gly Pro Gln Gly Pro Gly Gly Pro Pro Gly Pro Lys Gly Asn Ser
 260 265 270
 30 Gly Glu Pro Gly Ala Pro Gly Ser Lys Gly Asp Thr Gly Ala Lys Gly
 275 280 285
 Glu Pro Gly Pro Val Gly Val Gln Gly Pro Pro Gly Pro Ala Gly Glu
 290 295 300
 35 Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Pro Thr Gly Leu Pro
 305 310 315 320
 Gly Pro Pro Gly Glu Arg Gly Gly Pro Gly Ser Arg Gly Phe Pro Gly
 325 330 335
 40 Ala Asp Gly Val Ala Gly Pro Lys Gly Pro Ala Gly Glu Arg Gly Ser
 340 345 350
 Pro Gly Pro Ala Gly Pro Lys Gly Ser Pro Gly Glu Ala Gly Arg Pro
 355 360 365
 Gly Glu Ala Gly Leu Pro Gly Ala Lys Gly Leu Thr Gly Ser Pro Gly
 370 375 380
 45 Ser Pro Gly Pro Asp Gly Lys Thr Gly Pro Pro Gly Pro Ala Gly Gln
 385 390 395 400
 Asp Gly Arg Pro Gly Pro Pro Gly Pro Pro Gly Ala Arg Gly Gln Ala
 405 410 415
 50 Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Ala Gly Glu Pro Gly
 420 425 430
 Lys Ala Gly Glu Arg Gly Val Pro Gly Pro Pro Gly Ala Val Gly Pro
 435 440 445

55

Ala Gly Lys Asp Gly Glu Ala Gly Ala Gln Gly Pro Pro Gly Pro Ala
 450 455 460
 5 Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Pro Ala Gly Ser Pro Gly
 465 470 475 480
 Phe Gln Gly Leu Pro Gly Pro Ala Gly Pro Pro Gly Glu Ala Gly Lys
 485 490 495
 10 Pro Gly Glu Gln Gly Val Pro Gly Asp Leu Gly Ala Pro Gly Pro Ser
 500 505 510
 Gly Ala Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Val Gln Gly
 515 520 525
 15 Pro Pro Gly Pro Ala Gly Pro Arg Gly Ala Asn Gly Ala Pro Gly Asn
 530 535 540
 Asp Gly Ala Lys Gly Asp Ala Gly Ala Pro Gly Ala Pro Gly Ser Gln
 545 550 555 560
 20 Gly Ala Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala Gly
 565 570 575
 Leu Pro Gly Pro Lys Gly Asp Arg Gly Asp Ala Gly Pro Lys Gly Ala
 580 585 590
 25 Asp Gly Ser Pro Gly Lys Asp Gly Val Arg Gly Leu Thr Gly Pro Ile
 595 600 605
 Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Asp Lys Gly Glu Ser Gly
 610 615 620
 30 Pro Ser Gly Pro Ala Gly Pro Thr Gly Ala Arg Gly Ala Pro Gly Asp
 625 630 635 640
 Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro Pro
 645 650 655
 Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Pro Gly Asp Ala Gly
 660 665 670
 35 Ala Lys Gly Asp Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Pro
 675 680 685
 Pro Gly Pro Ile Gly Asn Val Gly Ala Pro Gly Ala Lys Gly Ala Arg
 690 695 700
 40 Gly Ser Ala Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly
 705 710 715 720
 Arg Val Gly Pro Pro Gly Pro Ser Gly Asn Ala Gly Pro Pro Gly Pro
 725 730 735
 45 Pro Gly Pro Ala Gly Lys Glu Gly Gly Lys Gly Pro Arg Gly Glu Thr
 740 745 750
 Gly Pro Ala Gly Arg Pro Gly Glu Val Gly Pro Pro Gly Pro Pro Gly
 755 760 765
 50 Pro Ala Gly Glu Lys Gly Ser Pro Gly Ala Asp Gly Pro Ala Gly Ala
 770 775 780
 Pro Gly Thr Pro Gly Pro Gln Gly Ile Ala Gly Gln Arg Gly Val Val
 785 790 795 800

55

5 Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly
 805 810 815
 Pro Ser Gly Glu Pro Gly Lys Gln Gly Pro Ser Gly Ala Ser Gly Glu
 820 825 830
 Arg Gly Pro Pro Gly Pro Met Gly Pro Pro Gly Leu Ala Gly Pro Pro
 835 840 845
 10 Gly Glu Ser Gly Arg Glu Gly Ala Pro Ala Ala Glu Gly Ser Pro Gly
 850 855 860
 Arg Asp Gly Ser Pro Gly Ala Lys Gly Asp Arg Gly Glu Thr Gly Pro
 865 870 875 880
 15 Ala Gly Pro Pro Gly Ala Xaa Gly Ala Xaa Gly Ala Pro Gly Pro Val
 885 890 895
 Gly Pro Ala Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro Ala Gly
 900 905 910
 20 Pro Ala Gly Pro Val Gly Pro Ala Gly Ala Arg Gly Pro Ala Gly Pro
 915 920 925
 Gln Gly Pro Arg Gly Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg
 930 935 940
 25 Gly Ile Lys Gly His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly
 945 950 955 960
 Pro Pro Gly Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro
 965 970 975
 30 Ala Gly Pro Arg Gly Pro Pro Gly Ser Ala Gly Ala Pro Gly Lys Asp
 980 985 990
 Gly Leu Asn Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly
 995 1000 1005
 35 Arg Thr Gly Asp Ala Gly Pro Val Gly Pro Pro Gly Pro Pro Gly Pro
 1010 1015 1020
 Pro Gly Pro Pro Gly Pro Pro Ser Ala Gly Phe Asp Phe Ser Phe Leu
 1025 1030 1035 1040
 Pro Gln Pro Pro Gln Glu Lys Ala His Asp Gly Gly Arg Tyr Tyr Arg
 1045 1050 1055
 40 Ala Arg Ser Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys
 1060 1065 1070
 Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly
 1075 1080 1085
 45 Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu
 1090 1095 1100
 Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val
 1105 1110 1115 1120
 50 Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys
 1125 1130 1135
 Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly
 1140 1145 1150

55

Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys
 1155 1160 1165

Lys Cys Ser
 1170

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1388 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln Leu Ser Tyr Gly Tyr Asp Glu Lys Ser Thr Gly Gly Ile Ser Val
 1 5 10 15
 Pro Gly Pro Met Gly Pro Ser Gly Pro Arg Gly Leu Pro Gly Pro Pro
 20 25 30
 Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Pro Pro Gly Glu Pro Gly
 35 40 45
 Glu Pro Gly Ala Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro
 50 55 60
 Pro Gly Lys Asn Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Arg Pro
 65 70 75 80
 Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Leu Pro Gly
 85 90 95
 Thr Ala Gly Leu Pro Gly Met Lys Gly His Arg Gly Phe Ser Gly Leu
 100 105 110
 Asp Gly Ala Lys Gly Asp Ala Gly Pro Ala Gly Pro Lys Gly Glu Pro
 115 120 125
 Gly Ser Pro Gly Glu Asn Gly Ala Pro Gly Gln Met Gly Pro Arg Gly
 130 135 140
 Leu Pro Gly Glu Arg Gly Arg Pro Gly Ala Pro Gly Pro Ala Gly Ala
 145 150 155 160
 Arg Gly Asn Asp Gly Ala Thr Gly Ala Ala Gly Pro Pro Gly Pro Thr
 165 170 175
 Gly Pro Ala Gly Pro Pro Gly Phe Pro Gly Ala Val Gly Ala Lys Gly
 180 185 190
 Glu Ala Gly Pro Gln Gly Pro Arg Gly Ser Glu Gly Pro Gln Gly Val
 195 200 205
 Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Ala Ala Gly Pro Ala
 210 215 220
 Gly Asn Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Ala Asn Gly
 225 230 235 240
 Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Ala Arg Gly Pro
 245 250 255

5 Ser Gly Pro Gln Gly Pro Gly Gly Pro Pro Gly Pro Lys Gly Asn Ser
 260 265 270
 Gly Glu Pro Gly Ala Pro Gly Ser Lys Gly Asp Thr Gly Ala Lys Gly
 275 280 285
 Glu Pro Gly Pro Val Gly Val Gln Gly Pro Pro Gly Pro Ala Gly Glu
 290 295 300
 10 Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Pro Thr Gly Leu Pro
 305 310 315 320
 Gly Pro Pro Gly Glu Arg Gly Gly Pro Gly Ser Arg Gly Phe Pro Gly
 325 330 335
 15 Ala Asp Gly Val Ala Gly Pro Lys Gly Pro Ala Gly Glu Arg Gly Ser
 340 345 350
 Pro Gly Pro Ala Gly Pro Lys Gly Ser Pro Gly Glu Ala Gly Arg Pro
 355 360 365
 20 Gly Glu Ala Gly Leu Pro Gly Ala Lys Gly Leu Thr Gly Ser Pro Gly
 370 375 380
 Ser Pro Gly Pro Asp Gly Lys Thr Gly Pro Pro Gly Pro Ala Gly Gln
 385 390 395 400
 25 Asp Gly Arg Pro Gly Pro Pro Gly Pro Pro Gly Ala Arg Gly Gln Ala
 405 410 415
 Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Ala Gly Glu Pro Gly
 420 425 430
 30 Lys Ala Gly Glu Arg Gly Val Pro Gly Pro Pro Gly Ala Val Gly Pro
 435 440 445
 Ala Gly Lys Asp Gly Glu Ala Gly Ala Gln Gly Pro Pro Gly Pro Ala
 450 455 460
 35 Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Pro Ala Gly Ser Pro Gly
 465 470 475 480
 Phe Gln Gly Leu Pro Gly Pro Ala Gly Pro Pro Gly Glu Ala Gly Lys
 485 490 495
 40 Pro Gly Glu Gln Gly Val Pro Gly Asp Leu Gly Ala Pro Gly Pro Ser
 500 505 510
 Gly Ala Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Val Gln Gly
 515 520 525
 Pro Pro Gly Pro Ala Gly Pro Arg Gly Ala Asn Gly Ala Pro Gly Asn
 530 535 540
 45 Asp Gly Ala Lys Gly Asp Ala Gly Ala Pro Gly Ala Pro Gly Ser Gln
 545 550 555 560
 Gly Ala Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala Gly
 565 570 575
 50 Leu Pro Gly Pro Lys Gly Asp Arg Gly Asp Ala Gly Pro Lys Gly Ala
 580 585 590
 Asp Gly Ser Pro Gly Lys Asp Gly Val Arg Gly Leu Thr Gly Pro Ile
 595 600 605

55

Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Asp Lys Gly Glu Ser Gly
 610 615 620
 5 Pro Ser Gly Pro Ala Gly Pro Thr Gly Ala Arg Gly Ala Pro Gly Asp
 625 630 635 640
 Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro Pro
 645 650 655
 10 Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Pro Gly Asp Ala Gly
 660 665 670
 Ala Lys Gly Asp Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Pro
 675 680 685
 15 Pro Gly Pro Ile Gly Asn Val Gly Ala Pro Gly Ala Lys Gly Ala Arg
 690 695 700
 Gly Ser Ala Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly
 705 710 715 720
 20 Arg Val Gly Pro Pro Gly Pro Ser Gly Asn Ala Gly Pro Pro Gly Pro
 725 730 735
 Pro Gly Pro Ala Gly Lys Glu Gly Gly Lys Gly Pro Arg Gly Glu Thr
 740 745 750
 25 Gly Pro Ala Gly Arg Pro Gly Glu Val Gly Pro Pro Gly Pro Pro Gly
 755 760 765
 Pro Ala Gly Glu Lys Gly Ser Pro Gly Ala Asp Gly Pro Ala Gly Ala
 770 775 780
 30 Pro Gly Thr Pro Gly Pro Gln Gly Ile Ala Gly Gln Arg Gly Val Val
 785 790 795 800
 Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly
 805 810 815
 35 Pro Ser Gly Glu Pro Gly Lys Gln Gly Pro Ser Gly Ala Ser Gly Glu
 820 825 830
 Arg Gly Pro Pro Gly Pro Met Gly Pro Pro Gly Leu Ala Gly Pro Pro
 835 840 845
 Gly Glu Ser Gly Arg Glu Gly Ala Pro Ala Ala Glu Gly Ser Pro Gly
 850 855 860
 40 Arg Asp Gly Ser Pro Gly Ala Lys Gly Asp Arg Gly Glu Thr Gly Pro
 865 870 875 880
 Ala Gly Pro Pro Gly Ala Xaa Gly Ala Xaa Gly Ala Pro Gly Pro Val
 885 890 895
 45 Gly Pro Ala Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro Ala Gly
 900 905 910
 Pro Ala Gly Pro Val Gly Pro Ala Gly Ala Arg Gly Pro Ala Gly Pro
 915 920 925
 50 Gln Gly Pro Arg Gly Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg
 930 935 940
 Gly Ile Lys Gly His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly
 945 950 955 960

55

5 Pro Pro Gly Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro
 965 970 975
 Ala Gly Pro Arg Gly Pro Pro Gly Ser Ala Gly Ala Pro Gly Lys Asp
 980 985 990
 Gly Leu Asn Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly
 995 1000 1005
 10 Arg Thr Gly Asp Ala Gly Pro Val Gly Pro Pro Gly Pro Pro Gly Pro
 1010 1015 1020
 Pro Gly Pro Pro Gly Pro Pro Ser Ala Gly Phe Asp Phe Ser Phe Leu
 1025 1030 1035 1040
 15 Pro Gln Pro Pro Gln Glu Lys Ala His Asp Arg Gly Arg Tyr Tyr Arg
 1045 1050 1055
 Ala Arg Ser Asp Glu Ala Ser Gly Ile Gly Pro Glu Val Pro Asp Asp
 1060 1065 1070
 20 Arg Asp Phe Glu Pro Ser Leu Gly Pro Val Cys Pro Phe Arg Cys Gln
 1075 1080 1085
 Cys His Leu Arg Val Val Gln Cys Ser Asp Leu Gly Leu Asp Lys Val
 1090 1095 1100
 25 Pro Lys Asp Leu Pro Pro Asp Thr Thr Leu Leu Asp Leu Gln Asn Asn
 1105 1110 1115 1120
 Lys Ile Thr Glu Ile Lys Asp Gly Asp Phe Lys Asn Leu Lys Asn Leu
 1125 1130 1135
 30 His Ala Leu Ile Leu Val Asn Asn Lys Ile Ser Lys Val Ser Pro Gly
 1140 1145 1150
 Ala Phe Thr Pro Leu Val Lys Leu Glu Arg Leu Tyr Leu Ser Lys Asn
 1155 1160 1165
 Gln Leu Lys Glu Leu Pro Glu Lys Met Pro Lys Thr Leu Gln Glu Leu
 1170 1175 1180
 35 Arg Ala His Glu Asn Glu Ile Thr Lys Val Arg Lys Val Thr Phe Asn
 1185 1190 1195 1200
 Gly Leu Asn Gln Met Ile Val Ile Glu Leu Gly Thr Asn Pro Leu Lys
 1205 1210 1215
 40 Ser Ser Gly Ile Glu Asn Gly Ala Phe Gln Gly Met Lys Lys Leu Ser
 1220 1225 1230
 Tyr Ile Arg Ile Ala Asp Thr Asn Ile Thr Ser Ile Pro Gln Gly Leu
 1235 1240 1245
 45 Pro Pro Ser Leu Thr Glu Leu His Leu Asp Gly Asn Lys Ile Ser Arg
 1250 1255 1260
 Val Asp Ala Ala Ser Leu Lys Gly Leu Asn Asn Leu Ala Lys Leu Gly
 1265 1270 1275 1280
 50 Leu Ser Phe Asn Ser Ile Ser Ala Val Asp Asn Gly Ser Leu Ala Asn
 1285 1290 1295
 Thr Pro His Leu Arg Glu Leu His Leu Asp Asn Asn Lys Leu Thr Arg
 1300 1305 1310

55

Val Pro Gly Gly Leu Ala Glu His Lys Tyr Ile Gln Val Val Tyr Leu
1315 1320 1325

His Asn Asn Asn Ile Ser Val Val Gly Ser Ser Asp Phe Cys Pro Pro
1330 1335 1340

Gly His Asn Thr Lys Lys Ala Ser Tyr Ser Gly Val Ser Leu Phe Ser
1345 1350 1355 1360

Asn Pro Val Gln Tyr Trp Glu Ile Gln Pro Ser Thr Phe Arg Cys Val
1365 1370 1375

Tyr Val Arg Ser Ala Ile Gln Leu Gly Asn Tyr Lys
1380 1385

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gln Leu Ser Tyr Gly Tyr Asp Glu Lys Ser Thr Gly Gly Ile Ser Val
1 5 10 15

Pro Gly Pro Met Gly Pro Ser Gly Pro Arg Gly Leu Pro Gly Pro Pro
20 25 30

Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Pro Pro Gly Glu Pro Gly
35 40 45

Glu Pro Gly Ala Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro
50 55 60

Pro Gly Lys Asn Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Arg Pro
65 70 75 80

Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Leu Pro Gly
85 90 95

Thr Ala Gly Leu Pro Gly Met Lys Gly His Arg Gly Phe Ser Gly Leu
100 105 110

Asp Gly Ala Lys Gly Asp Ala Gly Pro Ala Gly Pro Lys Gly Glu Pro
115 120 125

Gly Ser Pro Gly Glu Asn Gly Ala Pro Gly Gln Met Gly Pro Arg Gly
130 135 140

Leu Pro Gly Glu Arg Gly Arg Pro Gly Ala Pro Gly Pro Ala Gly Ala
145 150 155 160

Arg Gly Asn Asp Gly Ala Thr Gly Ala Ala Gly Pro Pro Gly Pro Thr
165 170 175

Gly Pro Ala Gly Pro Pro Gly Phe Pro Gly Ala Val Gly Ala Lys Gly
180 185 190

Glu Ala Gly Pro Gln Gly Pro Arg Gly Ser Glu Gly Pro Gln Gly Val
195 200 205

Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Ala Ala Gly Pro Ala

| | 210 | 215 | 220 |
|----|--------------------------------|------------------------------------|----------------------------|
| 5 | Gly Asn Pro Gly Ala Asp 225 | Gly Gln Pro Gly Ala Lys 230 | Gly Ala Asn Gly 235 240 |
| | Ala Pro Gly Ile Ala Gly 245 | Ala Pro Gly Phe Pro 250 | Gly Ala Arg Gly Pro 255 |
| 10 | Ser Gly Pro Gln Gly Pro 260 | Gly Gly Pro Pro Gly Pro 265 | Lys Gly Asn Ser 270 |
| | Gly Glu Pro Gly Ala Pro 275 | Gly Ser Lys Gly Asp Thr 280 285 | Gly Ala Lys Gly |
| 15 | Glu Pro Gly Pro Val Gly 290 | Val Gln Gly Pro Pro 295 | Gly Pro Ala Gly Glu 300 |
| | Glu Gly Lys Arg Gly Ala 305 | Arg Gly Glu Pro Gly 310 315 | Pro Thr Gly Leu Pro 320 |
| | Gly Pro Pro Gly Glu Arg 325 | Gly Gly Pro Gly Ser Arg 330 | Gly Phe Pro Gly 335 |
| 20 | Ala Asp Gly Val Ala Gly 340 | Pro Lys Gly Pro Ala Gly 345 | Glu Arg Gly Ser 350 |
| | Pro Gly Pro Ala Gly Pro 355 | Lys Gly Ser Pro Gly Glu 360 365 | Ala Gly Arg Pro |
| 25 | Gly Glu Ala Gly Leu Pro 370 | Gly Ala Lys Gly Leu Thr 375 | Gly Ser Pro Gly 380 |
| | Ser Pro Gly Pro Asp Gly 385 | Lys Thr Gly Pro Pro 390 395 | Gly Pro Ala Gly Gln 400 |
| 30 | Asp Gly Arg Pro Gly Pro 405 | Pro Gly Pro Pro Gly Ala 410 | Arg Gly Gln Ala 415 |
| | Gly Val Met Gly Phe Pro 420 | Gly Pro Lys Gly Ala Ala 425 | Gly Glu Pro Gly 430 |
| 35 | Lys Ala Gly Glu Arg Gly 435 | Val Pro Gly Pro Pro Gly 440 | Ala Val Gly Pro 445 |
| | Ala Gly Lys Asp Gly Glu 450 | Ala Gly Ala Gln Gly Pro 455 | Pro Pro Gly Pro Ala 460 |
| 40 | Gly Pro Ala Gly Glu Arg 465 | Gly Glu Gln Gly Pro Ala 470 475 | Gly Ser Pro Gly 480 |
| | Phe Gln Gly Leu Pro Gly 485 | Pro Ala Gly Pro Pro Gly 490 | Glu Ala Gly Lys 495 |
| 45 | Pro Gly Glu Gln Gly Val 500 | Pro Gly Asp Leu Gly Ala 505 | Pro Gly Pro Ser 510 |
| | Gly Ala Arg Gly Glu Arg 515 | Gly Phe Pro Gly Glu Arg 520 | Gly Val Gln Gly 525 |
| | Pro Pro Gly Pro Ala Gly 530 | Pro Arg Gly Ala Asn Gly 535 | Ala Pro Gly Asn 540 |
| 50 | Asp Gly Ala Lys Gly Asp 545 | Ala Gly Ala Pro Gly Ala 550 555 | Pro Gly Ser Gln 560 |
| | Gly Ala Pro Gly Leu Gln 565 | Gly Met Pro Gly Glu Arg 570 | Gly Ala Ala Gly |

55

| | 565 | 570 | 575 |
|----|--|-----|-----|
| 5 | Leu Pro Gly Pro Lys Gly Asp Arg Gly Asp Ala Gly Pro Lys Gly Ala 580 585 590 | | |
| | Asp Gly Ser Pro Gly Lys Asp Gly Val Arg Gly Leu Thr Gly Pro Ile 595 600 605 | | |
| 10 | Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Asp Lys Gly Glu Ser Gly 610 615 620 | | |
| | Pro Ser Gly Pro Ala Gly Pro Thr Gly Ala Arg Gly Ala Pro Gly Asp 625 630 635 640 | | |
| 15 | Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro Pro 645 650 655 | | |
| | Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Pro Gly Asp Ala Gly 660 665 670 | | |
| 20 | Ala Lys Gly Asp Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Pro 675 680 685 | | |
| | Pro Gly Pro Ile Gly Asn Val Gly Ala Pro Gly Ala Lys Gly Ala Arg 690 695 700 | | |
| 25 | Gly Ser Ala Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly 705 710 715 720 | | |
| | Arg Val Gly Pro Pro Gly Pro Ser Gly Asn Ala Gly Pro Pro Gly Pro 725 730 735 | | |
| 30 | Pro Gly Pro Ala Gly Lys Glu Gly Gly Lys Gly Pro Arg Gly Glu Thr 740 745 750 | | |
| | Gly Pro Ala Gly Arg Pro Gly Glu Val Gly Pro Pro Gly Pro Pro Gly 755 760 765 | | |
| 35 | Pro Ala Gly Glu Lys Gly Ser Pro Gly Ala Asp Gly Pro Ala Gly Ala 770 775 780 | | |
| | Pro Gly Thr Pro Gly Pro Gln Gly Ile Ala Gly Gln Arg Gly Val Val 785 790 795 800 | | |
| 40 | Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly 805 810 815 | | |
| | Pro Ser Gly Glu Pro Gly Lys Gln Gly Pro Ser Gly Ala Ser Gly Glu 820 825 830 | | |
| 45 | Arg Gly Pro Pro Gly Pro Met Gly Pro Pro Gly Leu Ala Gly Pro Pro 835 840 845 | | |
| | Gly Glu Ser Gly Arg Glu Gly Ala Pro Ala Ala Glu Gly Ser Pro Gly 850 855 860 | | |
| 50 | Arg Asp Gly Ser Pro Gly Ala Lys Gly Asp Arg Gly Glu Thr Gly Pro 865 870 875 880 | | |
| | Ala Gly Pro Pro Gly Ala Xaa Gly Ala Xaa Gly Ala Pro Gly Pro Val 885 890 895 | | |
| 55 | Gly Pro Ala Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro Ala Gly 900 905 910 | | |
| | Pro Ala Gly Pro Val Gly Pro Ala Gly Ala Arg Gly Pro Ala Gly Pro | | |

5 915 920 925
 Gln Gly Pro Arg Gly Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg
 930 935 940
 Gly Ile Lys Gly His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly
 945 950 955 960
 10 Pro Pro Gly Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro
 965 970 975
 Ala Gly Pro Arg Gly Pro Pro Gly Ser Ala Gly Ala Pro Gly Lys Asp
 15 980 985 990
 Gly Leu Asn Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly
 995 1000 1005
 20 Arg Thr Gly Asp Ala Gly Pro Val Gly Pro Pro Gly Pro Pro Gly Pro
 1010 1015 1020
 Pro Gly Pro Pro Gly Pro Pro Ser Ala Gly Phe Asp Phe Ser Phe Leu
 1025 1030 1035 1040
 25 Pro Gln Pro Pro Gln Glu Lys Ala His Asp Gly Gly Arg Tyr Tyr Arg
 1045 1050 1055
 Ala Arg Ser Pro Lys Asp Leu Pro Pro Asp Thr Thr Leu Leu Asp Leu
 1060 1065 1070
 30 Gln Asn Asn Lys Ile Thr Glu Ile Lys Asp Gly Asp Phe Lys Asn Leu
 1075 1080 1085
 Lys Asn Leu His Ala Leu Ile Leu Val Asn Asn Lys Ile Ser Lys Val
 35 1090 1095 1100
 Ser Pro Gly
 1105
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Claims

- 45 1. A chimeric DNA construct comprising a domain derived from a DNA sequence encoding a cellular regulatory factor and a domain derived from a DNA sequence encoding an extracellular matrix protein.
2. A chimeric DNA construct according to claim 1, wherein said extracellular matrix protein is selected from the group consisting of collagen, laminin, fibronectin, elastin and fibrin.
- 50 3. A chimeric DNA construct according to claim 1 or 2 wherein said cellular regulatory factor is selected from the group consisting of BMP, TGF- β , and decorin.
4. A chimeric DNA construct according to claim 1 or 2 wherein said cellular regulatory factor is selected from the group consisting of, a BMP fragment, a TGF- β fragment and a decorin peptide.
- 55 5. The DNA construct according to claim 3, wherein said BMP protein comprises BMP-2B.
6. A cloning vector comprising a DNA construct according to any one of claims 1 to 5.

7. A cloning vector according to claim 6, wherein said cloning vector is selected from the group consisting of plasmids, phages, cosmids and artificial chromosomes.
8. A cloning vector according to claim 6 or 7, wherein said cloning vector is pMal.
9. A cell transformed by a cloning vector according to any one of claims 6 to 8.
10. A cell according to claim 9 wherein said cell is selected from the group consisting of E. Coli, HeLa, 3T3, CHO, SP2, Sf9, Sf21, and High Five.
11. A chimeric protein comprising a domain derived from a cellular regulatory factor and a domain derived from an extracellular matrix protein.
12. A chimeric protein according to claim 11, wherein said extracellular matrix protein is selected from the group consisting of collagen, fibronectin, elastin, laminin and fibrin.
13. A chimeric protein according to claim 11 or 12, wherein said cellular regulatory factor is selected from the group consisting of BMP, TGF- β , decorin and a decorin peptide.
14. A method of manufacturing a chimeric cellular regulatory factor/extracellular matrix protein comprising: transforming a cell with the vector according to any one of claims 6 to 8; culturing said cell in a suitable culture medium; and obtaining said chimeric cellular regulatory factor/extracellular matrix protein from said culture medium.
15. A pharmaceutical vehicle for delivery of a therapeutically active substance comprising a chimeric protein having at least two domains, wherein one of said domains is at least a portion of an extracellular matrix protein and another of said domains is at least a portion of a therapeutically active moiety and said domains are covalently linked.
16. A pharmaceutical composition comprising a chimeric protein comprising a domain derived from a cellular regulatory factor and a domain derived from an extracellular matrix protein and a pharmaceutically acceptable vehicle.
17. A pharmaceutical composition according to claim 16, wherein said extracellular matrix protein is selected from the group consisting of collagen, fibronectin, elastin and fibrin.
18. A pharmaceutical composition according to claim 16 or 17, wherein said cellular regulatory factor is selected from the group consisting of BMP, TGF- β , decorin and a decorin peptide.
19. A pharmaceutical composition according to any one of claims 16 to 18, wherein said vehicle comprises a material selected from the group consisting of bioabsorbable polymers, biocompatible nonabsorbable polymers, lactone putty and plaster of Paris.
20. A pharmaceutical composition according to claim 19, wherein said material is selected from the group consisting of lactide, glycolide, trimethylene carbonate, dioxanone, caprolactone, polymethylmethacrylate and hydroxyethyl-methacrylate.
21. A method of preparing a DNA construct comprising: providing DNA which encodes a cellular regulatory factor or fragment thereof;
providing DNA which encodes an extracellular matrix protein or fragment thereof; and operably linking said cellular regulatory factor or fragment thereof encoding DNA to said extracellular matrix protein or fragment thereof encoding DNA to form a chimeric DNA construct.
22. Use of a chimeric protein according to any one of claims 11 to 13 or a pharmaceutical composition according to any one of claims 16 to 20 for the manufacture of a medicament for the prevention or treatment of disease.
23. Use of a chimeric protein according to claim 13 or a pharmaceutical composition according to any one of claims 18 to 20 for the manufacture of an osteogenic agent.
24. Use of a chimeric protein according to claim 13 or a pharmaceutical composition according to any one of claims 18 to 20, wherein said cellular regulatory factor is BMP, for the manufacture of a medicament for inducing bone and/or cartilage formation.

25. Use of a chimeric protein according to claim 13 or a pharmaceutical composition according to any one of claims 18 to 20, wherein said cellular regulatory factor is TGF- β , for the manufacture of a medicament for inducing soft tissue repair.

5 26. Use of a chimeric protein according to claim 13 or a pharmaceutical composition according to any one of claims 18 to 20, wherein said cellular regulatory factor is decorin or a decorin peptide for the manufacture of a medicament for reducing scar formation.

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(74) Representative: **Marsh, Roy David et al****Hoffmann Eitle & Partner****Patent- und Rechtsanwälte****Arabellastrasse 4****81925 München (DE)****(54) Recombinant chimeric proteins and methods of use thereof**

(57) A chimeric protein having at least one domain derived from a physiologically active moiety and at least one domain derived from an extracellular matrix protein is provided. Physiologically active domains are derived from physiologically active moieties such as bone morphogenic proteins, transforming growth factors, and dermatan sulfate proteoglycans. The extracellular matrix protein domains are derived from collagen, fibrin, fibro-gen, laminins and the like. Recombinant DNA constructs, cloning vectors and transformed cells containing DNA which encodes such chimeric proteins are provided. Methods of using the chimeric proteins, chimeric DNA constructs, cloning vectors containing chimeric DNA construct, and cells transformed with the cloning vectors are also provided. The chimeric proteins can be used as osteogenic agents and/or antiscarring agents.



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EUROPEAN SEARCH REPORT

Application Number
EP 95 10 9019

| DOCUMENTS CONSIDERED TO BE RELEVANT | | | |
|--|---|---|---|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
| X | US-A-5 302 701 (HASHI HIDETAKA ET AL) 12 April 1994 | 1,2,6,9, 11,12, 14-17, 21-23 | C12N15/62 C12N15/12 C07K14/51 C07K14/78 |
| Y | * the whole document * | 3-5,8, 10,13, 18-20 | C07K14/47 C07K14/495 A61K38/18 A61K38/39 C12N1/21 |
| Y | WO-A-90 03733 (INT GENETIC ENG) 19 April 1990 * claims 1,5,6,20,21 * | 3-5,13, 18-20,24 | //A61K47/48, (C12N1/21, C12R1:19) |
| Y | MOLECULAR ENDOCRINOLOGY, vol. 5, no. 1, pages 149-155, XP002000717 R.G.HAMMONDS ET AL.: "Bone-inducing activity of mature BMP-2b produced from a hybrid BMP-2a/2b precursor" * the whole document * | 3-5,8, 10,13,24 | |
| A | WO-A-94 01483 (COLLAGEN CORP) 20 January 1994 * claims 1-27 * | 3-5,8, 10,13,24 | TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07K C12N |
| The present search report has been drawn up for all claims | | | |
| Place of search THE HAGUE | | Date of completion of the search 20 December 1995 | Examiner Gurdjian, D |
| CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document | | T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document | |

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

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- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respects of which search fees have been paid, namely claims:
- ☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: mentioned in item 1.



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LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims 1-4,6-23 partially and 5,24 completely :
Chimeric proteins containing an extracellular matrix protein and a bone morphogenic protein.
2. Claims 1-4,6-23 partially and 25 completely :
Chimeric proteins containing an extracellular matrix protein and a transforming growth factor-beta.
3. Claims 1-4,6-23 partially and 26 completely :
Chimeric proteins containing an extracellular matrix protein and a decorin.